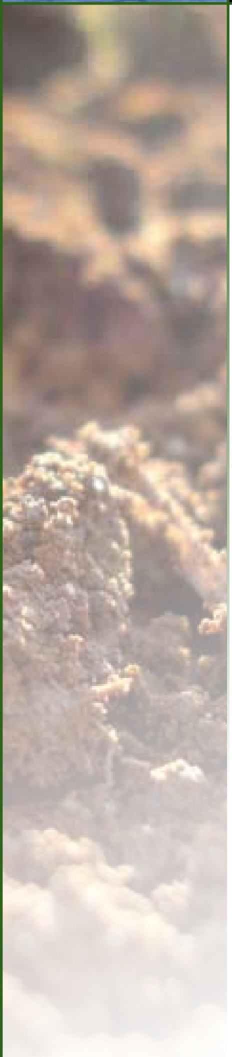
BIODIVERSITY & ECOSYSTEM SERVICES IN AGRICULTURAL PRODUCTION SYSTEMS



PERENNIAL CROPS FOR FOOD SECURITY

PROCEEDINGS OF THE FAO EXPERT WORKSHOP

28-30 August, 2013, Rome, Italy

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FOREWORD
TO THE PROCEEDINGS

Sustainable production systems have always relied on the flexibility, efficiency, and multiple functions of perennial trees and forages grown in combination with annual cereals, legumes, and oil species. But over the last 50 years, research, technologies and markets have focused mainly on a limited number of annual species to meet the increased demand for food. Furthermore, the primary focus was on increasing grain yields with reduced attention given to the social, environmental and market consequences of these food systems.

However, food security and agriculture are now entering an era characterized by scarce and depleted resources, climate change, price volatility and job losses. To adapt to this new era, agricultural technologies, science and markets have to be transformed to ensure sufficient food is produced for a growing population, while meeting simultaneously the economic, social and environmental challenges of twenty first century.

Perennial cereals, legumes and oil species represent a paradigm shift in agriculture and hold great potential to move towards sustainable production systems. Today, most agronomic practices used to grow annual crops require excessive water consumption, significant amounts of synthetic mineral fertilizers, labour, emissions of CO_2 and disrupt natural biological processes. Perennial crops instead are more rustic, improve soil structure and water retention capacity and contribute to increase climate change adaptation and mitigation practices and promote biodiversity and ecosystem functions.

Although in some ways perennial crops are at the forefront of scientific research with new varieties being developed, they also represent a thinking that goes back thousands of years when many cropping systems were based on perennial species including fruit trees, alfalfa, perennial rice, rye, and olive trees. In addition to modern breeding techniques, many wild and poorly domesticated species and varieties are available for research and interbreeding and hold potential to contribute to modern sustainable production systems. Through the development and breeding of these wild and semi-domesticated perennial varieties with commercially important and high yielding crops we will be able to achieve the best of both worlds.

Perennial crop research began in earnest about 30 years ago and has been growing ever since. There are now perennial crop varieties of oilseeds, legumes, wheat, sorghum, rice, sunflowers among many other crops. Significant uncertainties and challenges remain, related to increasing perennial crop yields and how to mainstream perennial crops into common farming practices and market systems.

FAO's Strategic Objectives are central to perennial crop research, specifically Strategic Objective 2 to: *Increase and improve provision of goods and services from agriculture, forestry and fisheries in a sustainable manner*. Perennial crops and the workshop were also developed under the framework of *Save and Grow* principles of ecological intensification of agricultural production.

CRA's strategic objectives are central to perennial crop research as well. In facts, CRA's mission is to perform agricultural research and develop innovation systems to alleviate poverty, increase food security and promote the sustainable use of natural resources, the same multiple objectives of perennial crops.

The proceedings of the Workshop held in FAO, 28-30 August 2013 organized by the Food and Agriculture Organization of the United Nations and the Consiglio per la Ricerca e la sperimentazione in Agricoltura (CRA) are intended to allow for the dissemination of the most recent research in the field. During the workshop gaps were identified, new partnerships discussed and priorities were identified for follow up actions.

The papers in these proceedings are arranged by the three main themes outlined during the workshop and include: *Genetics and breeding: state of the art, gaps and opportunities*; *Agro-systems, ecology and nutrition*; and *Policy, economics and way forward*. This was preceded by a welcoming address by Dr. Ren Wang, Assistant Director-General, Agriculture and Consumer Protection Department (AG), and followed by closing remarks by Dr. Clayton Campanhola, Director AGP.

The videos outline the main messages of the workshop by the participants and can be found at:

www.youtube.com/playlist?list=PLzp5NgJ2-dK4_itTMZqwUEg4BTBymkWgw, while material for the workshop can be found at:

www.fao.org/agriculture/crops/thematic-sitemap/theme/spi/fao-expert-workshop-on-perennial-crops-for-food-security/en/

We would like to thank the support for this workshop provided by FAO (especially Dr. Clayton Campanhola, Dr. Shakeel Bhatti, Dr. Constance Neely and Dr. Barbara Herren), the Consiglio per la Ricerca e la sperimentazione in Agricoltura (CRA) (especially Dr. Norberto Pogna, Dr. Stefano Bisoffi and Dr. Ida Marandola), the Land Institute (especially Dr. Stan Cox), Charles Sturt University (especially Dr. Len Wade) and CSIRO. We would like to thank all the presenters and participants at the workshop.

Joint signatures by:

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la sperimentazione in Agricoltura

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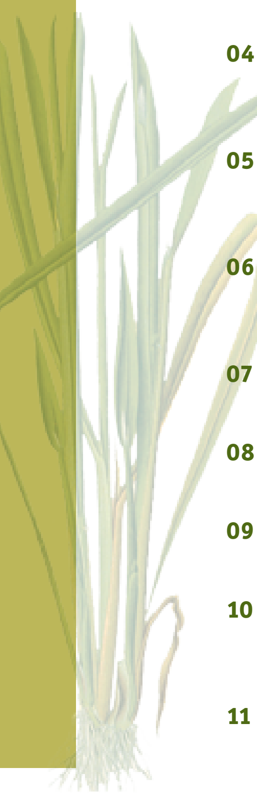

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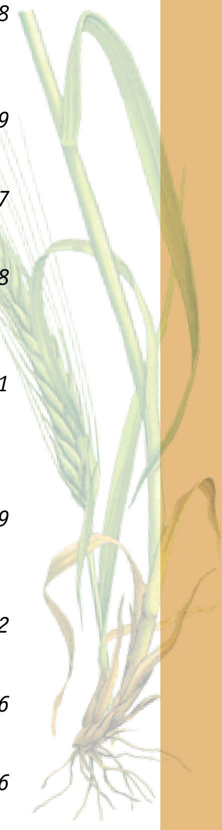

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LIST OF ABBREVIATIONS

ACIAR	Australian Centre for International Agricultural Research
AFLP	Amplified Fragment Length Polymorphism
AG	Agriculture and Consumer Protection Department
AGP	Plant Production and Protection Division
AGPM	Ecosystem Approach to Crop Production Intensification, Plant Production and Protection Division
AgriSA	Centre for Agriculture and Food Systems Analysis and Synthesis
AH	Agricultural Handbook
A-PAGE	Acidic Polyacrylamide Gel Electrophoresis
AR	5-n-alkylresorcinols
ArcGIS	Esri Geographic Information System software
ASEAN	Association of Southeast Asian Nations
ASL	Above Sea Level
CA	Conservation Agriculture
Ca	Calcium
CAPS	Cleaved Amplified Polymorphic Sequence
CAWT	Conservation Agriculture With Trees
CBOs	Community Based Organizations
CGIAR	Consultative Group on International Agricultural Research
CIMMYT	International Maize and Wheat Improvement Center
CP	Crude Protein Content
CPS	Maize/Pennycross/Soybean
CRA	Consiglio per la Ricerca e la sperimentazione in Agricoltura
CRC-PbMDS	Cooperative Research Centre for Plant-based Management of Dryland Salinity
CRC-FFI	Cooperative Research Centre for Future Farm Industries
CRS	Corn (maize)/Rye/Soybean
CS	Corn (maize)/Soybean
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CTAB	Cetyltrimethyl Ammonium Bromide
cv.	cultivar
cvs	cultivars
DF	Dietary Fibre
DM	Dry Matter
DNA	Deoxyribonucleic Acid
ERS	Economic Research Service
EST	Expressed Sequence Tags
FAO	Food and Agriculture Organization of the United Nations
GBS	Genotyping-By-Sequencing
GBSSI	Granule-Bound Starch-Synthase
GIS	Geographic Information System

GISH	Genomic *In Situ* Hybridization
GRDC	Grains Research and Development Corporation
GRIN	Germplasm Resources Information Network
GS	Genomic Selection
gSSURGO	Gridded Soil Survey Geographic
GWAS	Genome-Wide Association Studies
HMW-GS	High Molecular Weight-Glutenin Subunits
IAASTD	International Assessment of Agricultural Knowledge, Science and Technology for Development
ICRAF	World Agroforestry Centre
ICRISAT	The International Crops Research Institute for the Semi-Arid-Tropics
IRRI	The International Rice Research Institute
LD	Linkage Disequilibrium
LiDAR	Light Detection and Ranging
LMW-GS	Low Molecular Weight-Glutenin Subunits
MAS	Marker Assisted Selection
Mistra	Swedish Foundation for Strategic Environmental Research
MSTATC	Microcomputer Program for the Design, Management, and Analysis of Agronomic Research Experiments
NAD	Nicotinamide Adenine Dinucleotide
NERICA	New Rice for Africa
NGOs	Non-Governmental Organizations
NGS	Next Generation Sequencing
NLCD	National Land Cover Dataset
NRCS	Natural Resources Conservation Services
NSW DPI	New South Wales Department of Primary Industries
NUE	N-Uptake Efficiency
PCF	Protein Conversion Factor
PHR	Post-Harvest Regrowth
PIN-A	Puroindoline A
PIN-B	Puroindoline B
QTLs	Quantitative Trait Loci
RFLP	Restriction Fragment Length Polymorphism
R/qtl	an extensible, interactive environment for mapping quantitative trait loci
RNA	Ribonucleic Acid
RNA-seq	sequencing of Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
RS	Resistant Starch
RUSLE	Revised Universal Soil Loss Equation
UNEP	United Nations Environment Program
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis

SLU	Swedish University of Agricultural Sciences
SKCS	Single Kernel Characterization System
SNPs	Single-Nucleotide Polymorphisms
SP	Soluble Polyphenols
sp.	species (singular)
spp.	species (plural)
SSR	Simple-Sequence Repeats
TL	Tillers per plant
TNC	Total Non-structural Carbohydrate
UNEAK	Universal Network Enabled Analysis Kit
USDA	United States Department of Agriculture
UTM	Universal Transverse Mercator
WSU	Washington State University
YP	Yellow Pigments

INTRODUCTION

PERENNIAL CROPS FOR FOOD SECURITY

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Interest in breeding new perennial grain crops first arose in the early twentieth century, but it has been only in the past few years that the potential benefits of developing perennial grain-based cropping systems and the need to do so have become widely acknowledged. The subject is now drawing the attention of major scientific societies, leading journals, and governmental agencies. Much of that recent attention has been focused on the ecological benefits that communities of perennial plants can confer on a landscape: erosion prevention, efficient capture and use of water and nutrients, protection of water resources, carbon sequestration, and maintenance of thriving soil ecosystems. But with food security and rural livelihoods becoming an increasingly serious concern throughout the world, there is growing recognition of the potential benefits that intercropping of perennial grains offers smallholder farmers: reduced expenditure for seed, fertilizer, and other inputs; more reliable stand establishment and early vigour; less effort expended on weed control; extended growing seasons; less transplanting or other stoop labour, especially for women; and protection of biodiversity.

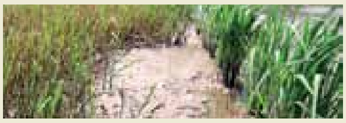
Perhaps the most important benefit of perennial agriculture will be the protection and development of healthy soil ecosystems that can ensure food security over the long term. That would achieve an important reversal of what is now an alarming trend. In 2011, the Food and Agriculture Organization (FAO) released its report The State of the World's Land and Water Resources for Food and Agriculture, concluding that 25 percent of the world's food-producing soils are highly degraded or are rapidly being degraded and that if moderately degraded soils are included, one-third of Earth's entire endowment of cropland is under threat. Loss of productive soil is most severe in the Himalayan and Andean regions; semi-arid tropical regions of Africa and India; rice-growing lands of Southeast Asia; and areas of intensive and industrialised farming throughout the world. Eighteen countries - nine of them in sub-Saharan Africa and four in Southeast Asia - now see more than half of their entire land area degrading rapidly. And while past production increases have received much of their impetus from irrigation, future freshwater resources are in at least as much trouble as the world's soils.

It is in this context that FAO, along with Italy's Council on Agricultural Research (CRA), Australia's Charles Sturt University, and The Land Institute in the United States, joined to host a meeting on Perennial Crops for Food Security in Rome in August, 2013. Seeing on the one hand the possibility that perennial crops can help address soil and water degradation, economic stresses, and malnutrition in food-insecure countries and on the other hand the emerging body of research on various aspects of perennial grain crops being produced by plant breeders, geneticists, agronomists, agro-ecologists, social scientists, and policy experts around the world, FAO determined that the time had come to bring together the key people involved in these disparate efforts. Forty-one people from ten nations participated in the meeting. The goals were to aggregate and put in context all research done on perennial grains up to now, begin forming a researchers' network, and plan for more extensive, well-coordinated and better-supported research in coming years. Essential to that effort will be drawing many more researchers and organizations into the perennial world.

The chapters that follow expand on the intense discussions that occurred in Rome. They provide a broad picture of the current state of perennial grain development and the diverse directions in which it is heading. Research on perennial cereals, grain legumes, and oilseeds, along with the cropping systems into which they will be assembled, can benefit from methods and technologies that have been well developed for staple grains grown currently. But to succeed, those methods must be supplemented by knowledge and experience, new and old, that applies uniquely to perennial crops. Domesticators and breeders of perennial grains have much to learn not only from farmers' experience but also from methods used in breeding woody perennials and perennial forage crops. Genomics research is already well-accustomed to moving across species boundaries, multiplying its possibilities. Meanwhile, working first with prototypes of perennial grains and later with improved lines and cultivars, agronomists, agro-ecologists, plant pathologists, and other researchers will face not only new challenges but also vast new opportunities to take advantage of natural processes that can improve and ensure food production. Grain quality and nutrition researchers, like all of the others mentioned above, will require significant input from farmers in the regions where the new perennial crops will be grown, as well as from social, economic, and policy analyses.

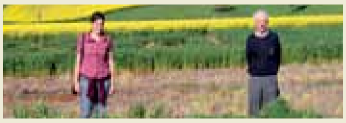
Emerging molecular-scale techniques have the potential to greatly improve the efficiency of perennial-grain breeding, but genotypic analyses cannot substitute for extensive phenotypic evaluations in diverse field locations. The three chief sets of traits that researchers are attempting to bring together—perenniality, productivity, and grain quality—are highly complex genetically, and they are strongly influenced by their environment. Relative expression of perenniality and other traits among genotypes is almost certain to vary widely over the diverse range of landscape positions, soils, climates, stresses, farming methods, and human preferences that perennial grains will encounter. Breeding populations must experience an adequate sample of all those factors, and must do so in the region where they are expected to be grown. The authors of this book take us on a tour of the ecological and human landscape where perennial grains currently grow and are being developed. Along the way, they relate how in their experience diverse scientific disciplines can converge to make perennial agriculture a mainstreamed reality. While this book furthers the exchange of knowledge and experience (and, one hopes, of plants and seed as well), its ultimate goal is to begin charting a course that will take perennial-grain research—which now consists of geographically and scientifically diverse, conceptually bold, but largely autonomous and independent projects—and weave them into a global network that can make this new agricultural concept a reality. In the interest of doing that, the final chapter attempts to lay out that course toward the new landscapes of the future.

01 Perennial crops: needs, perceptions, essentials

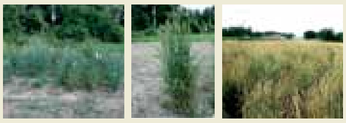


02 Perennial rice: challenges and opportunities

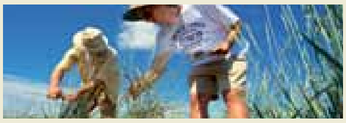
03 The progression of perennial rice breeding and genetics research in China



04 Perennial wheat breeding: current germplasm and a way forward for breeding and global cooperation

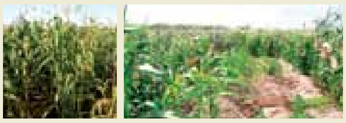


05 Evaluation of nine perennial wheat derivatives grown in Italy

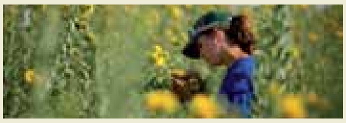


06 Current efforts to develop perennial wheat and domesticate *Thinopyrum intermedium* as a perennial grain

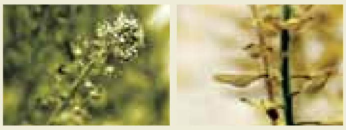
07 Viewpoint: multiple-harvest sorghums toward improved food security



08 Breeding and genetics of perennial maize: progress, opportunities and challenges

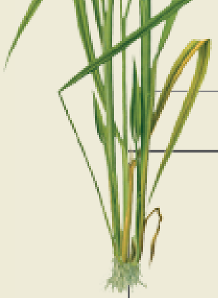


09 Evaluating perennial candidates for domestication: lessons from wild sunflower relatives



10 Domestication of *Lepidium campestre* as part of Mistra Biotech, a research programme focused on agro-biotechnology for sustainable food

11 Agriculture redesign through perennial grains: case studies



GENETICS AND BREEDING

STATE OF THE ART, GAPS AND OPPORTUNITIES

01

PERENNIAL CROPS:
NEEDS, PERCEPTIONS, ESSENTIALS

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ABSTRACT

The objective of this paper is to provide an overview of the essential issues for consideration in developing perennial crops. It is not intended to be a comprehensive review, rather it seeks to highlight topics that must be addressed in order to secure a strong future for perennial crops. To do so, the paper addresses a series of questions: Why do we need to develop perennial crops. What are the challenges and opportunities they provide. How do perceptions differ in the priority to develop perennial crops. What can be done to address and change these perceptions. How advanced is development of perennial crops. Two cases are then examined: perennial wheat in Australia as a case study for a developed country, and perennial rice in Asia as a case study for a developing country. Policy implications which may arise from development of perennial crops in developed and developing countries are then considered. The paper concludes by recognizing perennial crops may serve different purposes in different situations, so their roles must be carefully articulated. The issues raised are intended to be positive, meaning the perennial crops community should react positively to address them. The publication of hard evidence in scientific journals is considered essential, along with consideration and discussion of alternative views, in order to build credibility and confidence in the case for development and adoption of perennial crops in sustainable farming systems.

Keywords: perception, policy, publication, perennial rice, perennial wheat

INTRODUCTION

This paper is the first in the FAO Expert Workshop on Perennial Crops for Food Security. The intent of this viewpoint paper is to outline the essential issues for consideration in any decision to proceed to develop perennial crops. Essentially, this paper provides a road map of key considerations: Why do we need to develop perennial crops; What are the challenges and opportunities; What are the perceptions of perennial grains; What do we need to do to change those perceptions; Where are we up to in developing perennial crops; Perennial wheat in Australia as a case study for a developed country; Perennial rice in Asia as a case study for a developing country; What are the policy implications which may arise; Where will perennial crops be grown and what are the consequences; What do we need to do next; and some overall conclusions. Consequently, the paper is intended to flag the major issues for discussion, and to draw attention to a number of contributions later in the proceedings which deal with these topics in detail. This viewpoint is not intended to be a comprehensive review; rather it seeks to highlight topics that need to be addressed in order to secure a strong future for perennial grains. The issues raised are intended to be positive, meaning the perennial grains community should seek to address them.

WHY DO WE NEED PERENNIAL GRAINS?

Global population and demand for food are increasing, while arable land is limited and faces increasing risk of degradation. To ensure food and ecosystem security, development of perennial crops could provide more options under diverse and generally more marginal conditions (Glover *et al.* 2010a,b). Perennial crops should offer more stable surface cover against soil erosion, and improved nutrient balance against soil acidification, rising water tables and salinity, thereby improving ecosystem services. Systems which include perennial crops should also offer farmers greater flexibility and diversity of enterprise, including livestock, and greater stability of income. At different scales, the result should be improved farmer livelihood, improved ecosystem services, and improved food security (see Snapp *et al.* 2014; Runck, 2014; Snapp *et al.* 2014; Van den Putten, 2014; Reganold, 2014; Leakey, 2014, this volume).

WHAT ARE THE CHALLENGES AND OPPORTUNITIES THEY PROVIDE?

To be successful, perennial crops would need to be able to regrow after normal harvest, and able to retain floret fertility and set grain, despite wide hybridization with perennial species. The progeny would require selection for agronomic type, including plant height, flowering time, seed size, and non-shattering. Appropriate resistances would be important against disease, submergence, drought and soil constraints, depending on the characteristics of the target environment. Finally, the successful perennial crop would need to be compatible with its farming system (see Hayes *et al.* 2014; Bell, 2014; Dost, 2014, this volume).

HOW DO PERCEPTIONS DIFFER ON THE PRIORITY TO DEVELOP PERENNIAL GRAINS?

While we can see the benefits, many see problems, at least initially. The genetic challenges in wide crosses are complex, with reports of low seed set in amphiploids capable of regrowth after harvest (Cox *et al.* 2002). Consequently, some have dismissed prospects for the development of perennial crops as just too difficult.

Developed countries have expressed several concerns about potential threats to their established annual crops from perennial relatives. Given perennial species often have robust rhizomes, concern has been expressed that perennial crops may possess potential to become serious weeds, via hard-to-kill rhizomes. Many have been concerned with the possibility of the perennial providing a “green bridge” for disease, via the availability of living tissue able to propagate additional generations of disease, thereby increasing inoculum availability early in the season, encouraging earlier infection and increased risk of epidemics. These extra cycles of disease could enhance probabilities for mutations to bypass current plant resistance, thereby reducing the longevity of resistant cultivars. Finally, wide crosses are likely to result in shattering, small grains and reduced grain quality, necessitating separation of perennial grains from others, perhaps consigning them to feed grain only.

A different set of concerns may apply in developing countries, where the priority is food security, especially the availability of sufficient food for the family. The Consultative Group for International Agricultural Research (CGIAR) sees a need to prioritise increase in yield potential and closing the yield gap in high-yielding annual crops, especially under irrigation, to meet projected food demand. They discourage investment in perennial crops, as this may dilute yield gains. Such an analysis only considers grain supply, however, and not ecosystem services, such as minimising soil erosion, maintaining soil fertility, and providing diversified and stabilised systems contributing livestock as well for balanced nutrition.

At issue is a perceived trade-off with perennial crops (Connor *et al.*, 2011), which have to invest in perennial structures, at the expense of potentially directing that investment to further grain yield in the annual crop (see Cattani *et al.* 2014, this volume). Nevertheless, the additional investment in the perennial crop could result in increased acquisition of resources, which could compensate for their redeployment to perennial structures, or even result in yield gains overall (Glover, 2010; Glover *et al.* 2010a,b). There is a dearth of hard data on these issues, however, and it is essential that the perennial crops community address this.

There is also a wider imperative for developing perennial crops, in order not to cater only to those living and farming in productive irrigated areas. The green revolution neglected those remote from favourable ecosystems, and such input-dependent solutions had many pest and ecological concerns. Impact is needed in all farming systems, including the oft-neglected mixed farming systems in the diversified remote uplands of Asia, Africa and Latin America.

WHAT CAN BE DONE TO ADDRESS AND CHANGE THOSE PERCEPTIONS?

We need to show what these materials can do: ground cover, regrowth, floret fertility, forage dry matter (DM), resource capture, soil health, grain yield, disease, quality, biodiversity, ecosystem benefits, runoff, percolation, leaching. To do this well, we need to use a systems approach, and explore where the perennial crops would fit into the current and future farming systems.

Would the perennial displace the annual crop, or more likely, would it be preferentially suited to particular soils or situations, thereby contributing to the diversification and stability of the whole farming system and landscape. What is the plant requirement?? What traits should they possess?? What materials are available, and what are they capable of at this stage of development?? How do we go about improving them?? What are the next steps??

WHERE ARE WE UP TO IN DEVELOPING PERENNIAL CROPS?

As the papers in this volume attest, development of perennial crops ranges from its infancy (e.g. perennial maize and others, Murray and Jessop, 2014; Van Tassel *et al.,* 2014*;* Geleta *et al.,* 2014; Gross and Miller, 2014, this volume), to intermediate (e.g. perennial wheat, Jones *et al.,* 2014, this volume), to approaching reality (e.g. perennial sorghum, Paterson *et al., 2014;* Cox *et al., 2014;* Bozzini, 2014; perennial rice, Sacks *et al., 2014;* Hu *et al.,* 2014*;* Hill, 2014; and perennial legumes, Snapp *et al.,* 2014, this volume). The level of progress has depended in part on the duration of effort, and the complexity of genetic barriers encountered between domesticated and related wild species (Cox *et al.,* 2002). The latter issue has prompted the alternative approach of domesticating the wild species instead (e.g. *Thinopyrum intermedium*, Dehaan *et al.,* 2014, this volume). The diversity of species, approaches, and ecosystems targeted is positive, as we sort out what opportunities may arise. Nevertheless, as researchers and donors consider their strategies for the future, some targeting of investment by farmer demand, ecological need, breeding progress, and policy implication is likely (see below, and also Dixon and Garrity, 2014, this volume).

Further, sustained progress and the development of perennial crops targeted to farmer needs is likely to require systems approaches, in order to assist compatibility with farmer practice and the likelihood of adoption. To explore this, and to set up a basis for examining policy implications, two cases are examined: perennial wheat in Australia as a case study for a developed country, and perennial rice in Asia as a case study for a developing country.

PERENNIAL WHEAT IN AUSTRALIA AS A CASE STUDY FOR A DEVELOPED COUNTRY

An example of the use of the systems approach is provided by research in perennial wheat in Australia, supported by related efforts in the United States of America. Bell *et al.* (2008) used MIDAS, a bioeconomic model of a mixed crop-livestock farming system to explore what role perennial wheat may play in the farming system. Perennial wheat used solely for grain production was not selected as part of an optimal farm plan under the standard assumptions. In contrast, dual-purpose perennial wheat that produces grain and additional forage during summer and autumn could increase farm profitability substantially (AU$20/ha over the whole farm) and 20 percent of farm area was selected for perennial wheat production on the optimal farm plan under the standard assumptions. As little as 800 kg/ha of forage from perennial wheat could reduce demands on stubble over summer and grain supplement at break of season and increase farm stock numbers. The additional value of this timely grazing reduced the relative yield required for perennial wheat to be profitable to just 40 percent of that of the annual wheat crop. This analysis suggested that dual-purpose perennial wheat would be a profitable option for mixed crop/livestock farmers.

The challenges in developing perennial wheat for Australia were reviewed by Bell *et al.* (2010), following ground-breaking work in the United States to generate amphiploids between annual *Triticum* and perennial *Thinopyrum* species (Cox *et al.* 2010; Murphy *et al.* 2010). Experimental efforts in Australia commenced by evaluating a diverse array of putative perennial wheat derivatives including germplasm imported from the United States perennial wheat programs, Russian and Chinese wide-cross germplasm and assorted lines from the Australian Wheat Collection. This initial evaluation of over 150 wheat x wheatgrass derivatives assessed capacity to regrow post-harvest, and yield grain over successive years, thereby identifying characteristics common to surviving breeding lines (Hayes *et al.* 2012; Jaikumar *et al.* 2012). Several entries persisted to produce grain over three successive years. Regrowth was associated with the presence of at least one whole genome equivalent (14 chromosomes) from the perennial donor species. This research established that developing a perennial wheat may be feasible, even though existing germplasm was not intended for Australian conditions, nor was it yet sufficiently developed to be deployed commercially.

The research continues with further field evaluations of perennial wheat derivatives, including previously untested germplasm, and an additional fourth year of those entries surviving from the initial report. In addition, three more-detailed experiments were examined, which used a common set of six genotypes to evaluate forage biomass production under serial defoliation, changes in root-shoot partitioning of DM over successive regrowth cycles, and dehydration tolerance and plant survival under severe water deficit and re-watering. Based on this evidence, a breeding approach for developing adapted perennial wheat for Australian farmers has been proposed (Larkin *et al.* 2014; Larkin and Newell, 2014, this volume).

Further, this research establishes a need to pursue mechanistic understanding in order to make sustained progress. Some of the United States material is reported to be perennial in the glasshouse, but fails to survive in the field. When grown in Australia, however, some of this material has survived and regrown in the field for three seasons. Is this due to the severity of abiotic stresses encountered, such as cold and snow cover in winter, and severe drought and high temperature in summer. Is survival due to better agronomy such as rotation with Brassicas, a different soil or its key attributes such as pH or drainage, or is it related to tolerance to particular diseases such as root and crown rots. By understanding why materials fail in different situations, sustained breeding and agronomic progress is more likely to result.

PERENNIAL RICE IN ASIA AS A CASE STUDY FOR A DEVELOPING COUNTRY

A second case study is drawn from perennial rice in Asia. Perennial rice was originally proposed as a plant type to improve soil stability on sloping uplands, while contributing forage for livestock and grain for the farmer. Initially, crosses were made between *Oryza sativa* and *Oryza longistaminata*, and between *Oryza sativa* and *Oryza rufipogon* (Sacks *et al.* 2014, this volume), which provided sources of nematode and drought resistance from the wild species. The materials were passed to Yunnan Academy of Agricultural Sciences in Kunming China, where Professor Fengyi Hu and his team continued the breeding effort with great success. By selection and repeated backcrossing, they were able to increase spikelet fertility while retaining perennial traits allowing regrowth. Suitable plant types for favourable lowland environments have resulted, with one entry, PR23, now in pre-release testing in Yunnan province. In addition, two QTL for rhizome development have been identified and sequenced (Hu *et al.* 2014, this volume). Breeding and genetic progress in perennial rice has been impressive, but the associated understanding of target environments, and how the materials generated perform in them, is at its early stages, though is now being addressed in field experiments in Yunnan Province in China, and in Savanakhet and Champassak Provinces in neighbouring Lao PDR (Wade and Sengxua, 2014). There is a need to build on this collaboration to further strengthen the characterisation, agronomy, physiology and field testing in association with the breeding program, to ensure sustained progress in the future. In particular, success in addressing the original target, perennial rice for the drought-prone rainfed lowland and upland environments, will require additional investment and collaboration, especially for perennial survival and regrowth in harsh dry conditions.

WHAT ARE THE POLICY IMPLICATIONS FOR THE DEVELOPED COUNTRY?

In the developed country, high priority was allocated to protection of the established annual crop, its production and marketing system. At issue is concern with disease, weediness or poor grain quality. Any proposed breeding effort must ensure levels of disease resistance at least equal to currently released cultivars, for the most common diseases such as stem rust, leaf rust, stripe rust, and septorias. The intent is to ensure the perennial crop could not act as an out-of-season stepping stone to development of spore epidemics, nor to encourage mutation and breakdown of resistance of useful genes. To an extent, the risk here may be less than envisaged, as there is unlikely to be a large green canopy on the perennial crop when soil water is limiting after harvest. The perennial nature, however, may render certain diseases even more important than in the annual crop. Examples include insect-transmitted viruses such as wheat streak mosaic virus and barley yellow dwarf virus. Nevertheless, the wild progenitors possess strong resistance to these viruses, which the evidence suggests is passed to the progeny. For Australia, the root and crown rots may be a special threat for perennials, with the intention to grow the perennial crop for several seasons. Here, crop rotation with brassicas and soil health will be important to ensure levels of infection are initially low, as resistance is not strong against many root and crown rots. Nevertheless, it will be important to include the best resistance available for these critical diseases. To address these concerns, the developed country may require specified levels of resistance to particular diseases, or require the perennial only be grown for a specified number of seasons before rotating to another crop.

The perennial parent of many of the perennial wheats, *Thinopyrum intermedium*, is a prohibited species in Australia, requiring stringent quarantine procedures before the perennial wheats could be grown in the field in Australia. The concern was robust or long rhizomes, which may be hard to kill, and which could pose a significant weediness threat. Fortunately, the introduced lines did not possess rhizomes, and their regrowth was in the form of tillers in the next cycle from the crown of the plant. Hence the risk of weediness was greatly reduced, but it is still worthwhile checking progeny to make sure. To address this, the developed country may require that released perennial wheats only regrow as tillers not rhizomes.

Grain quality is a critical element of successful marketing of Australian wheat to meet quality requirements for a number of carefully defined markets. Grain is delivered to separate silos, and handled and marketed separately, to ensure consistent quality. Were a lower grain quality perennial wheat to be released, its grain would need to be segregated, perhaps as feed wheat only. Such arrangements are already in place, so should be acceptable, though no doubt there would be concern if larger quantities of low quality wheat were delivered, relative to the more desirable high-quality, higher-priced grain. To address this concern, grain must be segregated by quality and sufficient facilities must be available to cope, until grains of comparable quality become available. The importance of grain quality, and the associated benefits to human health, is considered in several papers in this proceeding (Pogna *et al.* 2014; Sands *et al.* 2014, this volume).

WHAT ARE THE POLICY IMPLICATIONS FOR THE DEVELOPING COUNTRY?

To an extent, the issues for the developing country may include aspects of those for the developed country above, but the contrast may relate to grain for export versus feed for the family. In the developing country, food security and livelihood for the farmer and the family are paramount, especially assurance of food supplies until the next harvest. Governments in developing countries may be more willing to explore opportunities, to see what benefits could accrue for subsistence farmers, and those governments are very concerned with sustained production. Hence, developing countries may more readily recognize the potential opportunities with perennial crops and allow farmers to explore them.

WHERE WILL PERENNIAL CROPS BE GROWN AND WHAT WILL BE THE CONSEQUENCES?

The discussion above is designed to draw attention to the reality that perennial crops are unlikely to soon replace high-yielding annual crops as the mainstay of grain production. More likely, they will fulfil niche roles across the landscape, perhaps being selected to stabilise land intermediate between prime cropping and grazing only, especially if some soil remediation is needed. As such, a perennial crop is most likely to be dual purpose, including provision of timely feed to livestock. The analyses of Bell *et al.* (2008) and Pimentel *et al.* (2012) provide examples of the potential roles of perennial wheat. Likewise, mountain uplands may provide a scenario ideal for inclusion of perennial rice. The lowland paddy at altitude would still support high-yielding annual rice and other crops, but the surrounding sloping uplands, where upland rice is normally grown, may be ideal for perennial rice for grain and grazing, perhaps in conjunction with either agroforestry or self-regenerating annual legumes for further diversification and system stability. Another example is intercropping of annual peanut and perennial pigeon pea in Malawi (Snapp *et al.* 2014 this volume), to provide not only feed for farmer and livestock, but also nitrogen to a following maize crop. In each of these examples, the landscape is improved, with a more productive and stable multi-purpose farming system.

WHAT WE NEED TO DO NEXT

The most critical issue facing the perennial crops community is to build credibility and confidence in the broader scientific population, and especially in the donor community. To do this, we need to collect and publish hard data on the performance of perennial crops, and especially on tradeoffs in performance, benefits to resource capture and timeliness, and alternative systems benefits such as via livestock or ecosystem services. Previously, publications from the perennial crops community tended to be longer on exciting concepts, but shorter on evidence to demonstrate what they could actually deliver. Some publication streams are now in progress to address this, but further effort is needed. I believe donor success is related not only to concept development in exciting proposals, but also to hard evidence and strong track records of delivery. Given these are questions about the validity of developing perennial grains, e.g. yield tradeoffs, grain security, we must assemble and publish evidence to demonstrate the viability of our visions, and also, a likely time frame to their achievement. This workshop is an ideal foundation for a coordinated effort to develop perennial crops, based on sound scientific evidence, relevance to farmer needs, and in a manner compatible with sustainable and profitable farming systems (see also Snapp *et al.* 2014 and final chapter, this volume).

CONCLUSIONS

Prospects for developing perennial crops are bright, especially if efforts are targeted to appropriate environmental niches with appropriate species. For example, the Grains Research and Development Corporation has recently listed perennial wheat as a technology on the horizon, which has potential to contribute to the next substantial advance in Australia. Perennial rice line PR23 is now in pre-release testing in the Yunnan Province of China, raising prospects of commercial release to farmers shortly. Annual peanut-perennial pigeon pea-maize is already being used by farmers in Malawi. Hence, opportunities to include perennial crops in productive, stable and sustainable dual-purpose systems are appearing already. We need to continue our efforts and document our progress as we proceed in order to ensure support of scientific and donor communities towards a perennial cropping systems future.

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REFERENCES

Bell, L.W., Byrne, F., Ewing, M. & Wade, L.J. 2008. A preliminary whole-farm economic analysis of perennial wheat in an Australian farming system. *Agriculture Systems*. 96: 166-174.

Bell, L.W., Wade, L.J. & Ewing, M.A. 2010. Perennial wheat: a review of environmental and agronomic prospects for development in Australia. *Crop Pasture Science*. 61: 679-690.

Connor, D.J., Loomis, R.S. & Cassman, K.G. 2011. *Crop Ecology: Productivity and Management in Agricultural Systems*, 2nd Edition. Cambridge Univ. Press, 568 p.

Cox, T.S., Bender, M., Picone, C., Van Tassel, D.L., Holland, J.B., Brummer, E.C., Zoeller, B.E., Paterson, A.H. & Jackson, W. 2002. Breeding perennial grain crops. *Critical Reviews in Plant Sciences*. 21: 59-91.

Cox, T.S., Van Tassel, D.L., Cox, C.M. & Dehaan, L.R. 2010. Progress in breeding perennial grains. *Crop Pasture Science*. 61: 513-521.

Glover, J.D. 2010. Editorial: Harvested perennial grasslands: Ecological models for farming's perennial future. *Agriculture Ecosystems and Environment*. 137: 1-2.

Glover, J.D., Reganold, J.P., Bell, L.W., Borevitz, J., Brummer, E.C., Buckler, E.S., Cox, C.M., Cox, T.S., Crews, T.E., Culman, S.W., Dehaan, L.R., Eriksson, D., Gill, B.S., Holland, J., Hu, F., Hulke, B.S., Ibrahim, A.M.H., Jackson, W., Jones, S.S., Murray, S.C., Paterson, A.H., Ploschuk, E., Sacks, E.J., Snapp, S., Tao, D., Van Tassel, D.L., Wade, L.J., Wyse, D.L. & Xu, Y. 2010a. Increasing food and ecosystem security via perennial grains. *Science*. 328: 1638-1639.

Glover, J.D., Reganold, J.P., Bell, L.W., Borevitz, J., Brummer, E.C., Buckler, E.S., Cox, C.M., Cox, T.S., Crews, T.E., Culman, S.W., Dehaan, L.R., Eriksson, D., Gill, B.S., Holland, J., Hu, F., Hulke, B.S., Ibrahim, A.M.H., Jackson, W., Jones, S.S., Murray, S.C., Paterson, A.H., Ploschuk, E., Sacks, E.J., Snapp, S., Tao, D., Van Tassel, D.L., Wade, L.J., Wyse, D.L. & Xu, Y. 2010b. Perennial questions of hydrology and climate. *Science*. 330: 33-34.

Hayes, R.C., Newell, M.T., Dehaan, L.R., Murphy, K.M., Crane, S., Norton, M.R., Wade, L.J., Newberry, M., Fahim, M., Jones, S.S., Cox, T.S. & Larkin, P.J. 2012. Perennial cereal crops: An initial evaluation using wheat derivatives. *Field Crops Research*. 133: 68-89.

Jaikumar, N.S., Snapp, S.S., Murphy, K. & Jones, S.S. 2012. Agronomic assessment of perennial wheat and perennial rye as cereal crops. *Agronomy Journal*. 104: 1716-1726.

Larkin, P.J., Newell, M.T., Hayes, R.C., Aktar, J., Norton, M.R., Moroni, S.J. & Wade, L.J. 2014. Progress in developing perennial wheats for grain and grazing. *Crop Pasture Science*. http://dx.doi.org/10.1071/CP13330.

Murphy, K.M., Lyon, S.R., Balow, K.A. & Jones, S.S. 2010. Post-sexual cycle regrowth and grain yield in *Thinopyrum elongatum* x *Triticum aestivum* amphiploids. *Plant Breeding*. 129: 480-483.

Pimentel, D., Cerasale, D., Stanley, R.C., Perlman, R., Newman, E.M., Brent, L.C., Mullan, A. & Tai-I Chang, D. 2012. Annual vs perennial grain production. *Agriculture Ecosystems and Environment*. 161: 1-9.

Wade, L.J. & Sengxua, P. 2014. Developing improved farming and marketing systems in rainfed regions of southern Lao PDR. *Lao Journal of Agriculture and Forestry*. (In press).

02

PERENNIAL RICE: CHALLENGES AND OPPORTUNITIES

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ABSTRACT

As the human population continues to grow, and governments increasingly incentivize the migration of people from rural areas to cities, there is pressure to produce more food with fewer resources, including water, fertilizer, and especially labour. The development of high-yielding, perennial cultivars of rice could help meet the need for more food while reducing the inputs required. Domesticated Asian rice (*Oryza sativa*) was derived from perennial ancestors. Moreover, the traditional practice of ratoon cropping demonstrates that domesticated Asian rice retains some of its original perennial character, though this varies greatly by cultivar. However, with currently available cultivars, the yields of first ratoon crops are typically 40 percent or less of main crop yields and subsequent decreases make further cycles of ratooning uneconomical. Thus, the key research and development challenges for perennial rice are to improve ratoon yields, increase longevity, and improve drought tolerance with the goal of facilitating perennial rice regrowth and production beyond irrigated systems and into seasonally dry upland and rainfed lowland environments. Opportunities (in order of increasing difficulty and potential gain) for developing high-yielding cultivars of perennial rice include: 1) breeding within domesticated Asian rice germplasm for improved ratooning ability and yield, 2) introgressing genes from the perennial, rhizomatous A-genome species *O. longistaminata*, 3) domesticating the rhizomatous species *O. longistaminata* and *O. australiensis*.

Keywords: perennial rice, *Oryza sativa*, *O. longistaminata*, ratoon, introgression

INTRODUCTION

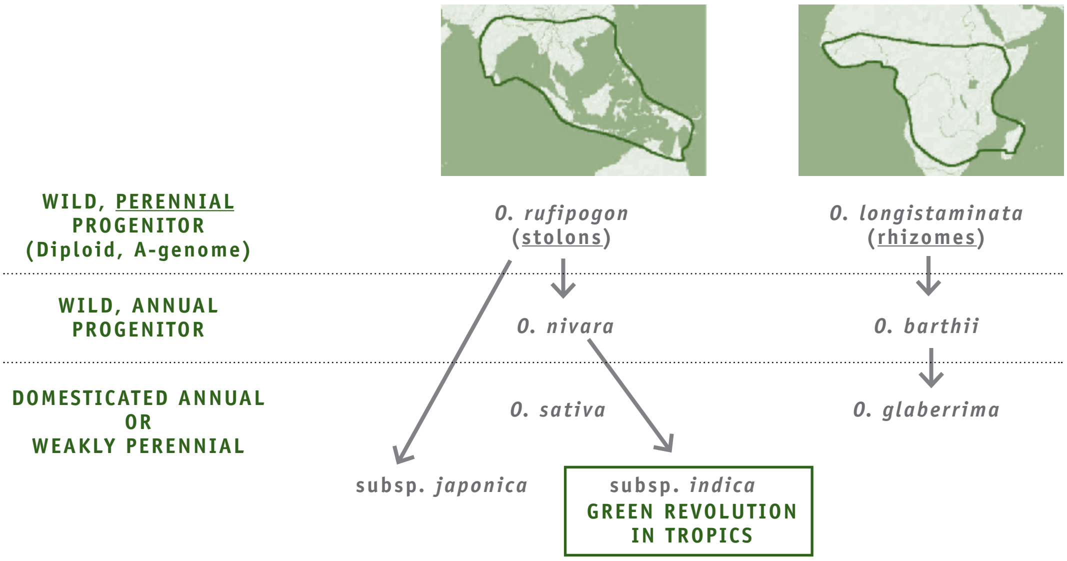
Many rice cultivars grown today are perennials that are cultivated as annuals (Figure 1). Genotypes of *Oryza sativa* vary greatly in their propensity to ratoon (regrow) after an initial harvest of grain (Chauhan *et al.* 1985; Krishnamurthy, 1988). Ratoon cropping of rice was a traditional practice, especially in East Asia (Hill, 2010), that became less common during the second half of the twentieth century. However, a notable modern exception has been an emphasis on ratoon crop production in the United States Gulf Coast to increase overall seasonal yields (Bollich and Turner, 1988), which is an economically viable strategy where warm weather persists long enough for a ratoon crop but is insufficient in duration for a new planting from seed to mature.

FIGURE 1. A RICE FIELD IN JAPAN DURING AUTUMN SHOWS VIGOROUS REGROWTH AFTER HARVEST OF THE FIRST CROP

Cool weather prevents development and harvest of a ratoon grain crop at this location.

Both species of domesticated rice, *O. sativa* from Asia and *O. glaberrima* from Africa, are derived from perennial ancestors, either directly or via an annual intermediary (Figure 2). Cheng *et al.* (2003) found that the progenitor species of *O. sativa*, *O. rufipogon*, consisted of four major clades, three of which were perennial and one of which was annual (the annual clade is sometimes referred to as *O. nivara*). Moreover, Cheng *et al.* (2003) found that the *indica* subspecies of *O. sativa* was derived from the annual *O. rufipogon* clade and that the *japonica* subspecies was derived from one of the perennial *O. rufipogon* clades. Though there is currently much debate about whether *O. sativa* was derived from a single or multiple domestications, the results of Cheng *et al.* (2003) are consistent with observations of some workers that *japonica* cultivars are typically better than *indica* cultivars for ratooning (Shahi and Raharinirian, 1988) and as parents for breeding strongly perennial rice (Sacks *et al.* 2007). In the Southern United States, which is the primary example of successful ratoon cropping of rice on a large commercial scale, production is based on tropical *japonica* cultivars (Lu *et al.* 2004). Recently, New Rice for Africa (NERICA) cultivars and their japonica parent were found to have higher ratoon yields under upland conditions than their *O. glaberrima* parent (Sanni *et al.* 2009). However, some of the green revolution *indica* cultivars, such as IR8 and IR64, have also been observed to ratoon well (Chauhan *et al.* 1985; Munda *et al.* 2009). In any case, it is clear that there is considerable genetic diversity within *O. sativa* for perennial growth, and additional diversity is present in its wild relatives, *O. rufipogon* and *O. longistaminata*. Perennation in *O. sativa* is by growth of axillary buds on older tillers (i.e. tillering), whereas *O. rufipogon* can additionally propagate from stolons, and *O. longistaminata* produces many long rhizomes that are the primary source of new shoots.

FIGURE 2. ORIGINS OF DOMESTICATED RICE IN RELATION TO PERENNIAL GROWTH



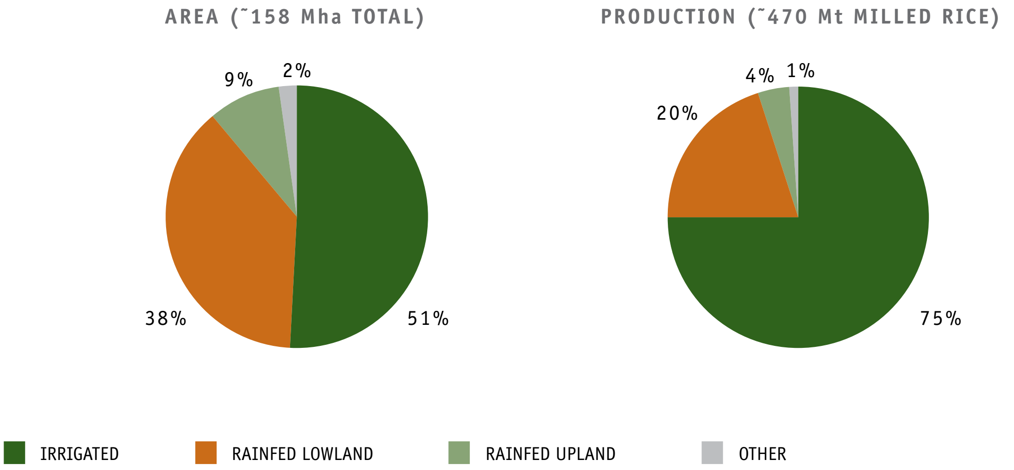
Source: http://www.knowledgebank.irri.org/extension/wild-rice-taxonomy.html

PROBLEMS THAT PERENNIAL RICE COULD HELP SOLVE

Rice is a critically important staple food and the demand for more production is expected to continue to increase, especially in developing countries. Deployment of perennial rice could meet important needs, such as increased production per growing season, reduced risks for farmers, lower labour requirements, less water needed, and protecting soil from erosion.

Irrigated rice accounts for 51 percent of the area planted to rice but 75 percent of rice production (Figure 3). In contrast, rainfed lowland rice accounts for 38 percent of the area but just 20 percent of production, and rainfed upland rice accounts for 9 percent of rice lands but only 4 percent of production (Figure 3). Thus, a major challenge for improving availability of rice is to increase production in rainfed systems. Where rainfed fields in tropical monsoonal environments cannot be converted to irrigated, a potentially valuable strategy for increasing production is to maximize use of rainfall via a ratoon crop. In contrast to annual rice cultivation, ratoon rice could take advantage of end-of-season moisture in a typical year, and also be positioned for even greater yields in wet years. Such a use of season-limiting moisture would be analogous to the use of ratooning in the Southern United States of America to take advantage of end-of-season warmth. Cultivars with tolerance to drought, in addition to high ratooning ability would be especially useful for rainfed production of a ratoon crop. By modifying crop duration from a single long-season crop to a medium duration main crop plus a short-medium duration ratoon crop, it may also be possible to reduce production risks associated with variable abiotic and biotic stress pressures. Adoption of early maturing rice cultivars was critical to the establishment of ratooning in the Southern United States of America (Bollich and Turner, 1988).

FIGURE 3. RICE AREA AND PRODUCTION BY CULTIVATION SYSTEM



Source: http://irri.org/index.php?option=com_k2&view=item&layout=item&id=9151&lang=en

Field preparation and planting of paddy rice, typically via initial establishment of seedbeds followed by transplanting of seedlings, is labour intensive. For upland rice, planting and especially weeding can require a major investment of labour. For example, in Central and West Africa, upland rice accounts for ~40 percent of the rice area, but employs ~70 percent of the region's rice farmers (http://irri.org/). As governments increasingly incentivize urbanization, the availability of labour for farm work will continue to be reduced. Thus, there is a great and increasing need to maximize production of rice per unit of labour. Perennial rice enables multiple harvests from a single planting, thereby improving labour efficiency. Though improvements in labour efficiency may be expected via mechanization, such gains come with capital costs that are often too great for many farmers in developing countries, whereas seed of improved perennial cultivars can be made available for little or no additional cost to farmers.

Hybrid rice has brought yield benefits to farmers who can afford to invest in this technology. However, hybrid rice seed is expensive to produce and thus is sold at a premium. If additional production in farmers' fields can be obtained from hybrid rice crops via ratooning, then the return on investment for a farmer would be greater (Bollich and Turner, 1988; de la Rosa, 2004) and this advantage could make it economically feasible for more farmers to adopt.

Water is a major input for rice production. Global climate change is expected to negatively impact availability of water for rice production by reducing winter snow-packs on mountains and by the melting of glaciers that feed rivers used to supply irrigation water for rice fields. It is well established that ratoon crops of rice require less water than main crops, thereby improving the overall efficiency of water use for rice production. Thus, increasing production of rice via cultivating more ratoon crops and increasing ratoon yields would further improve efficiency of water use.

Upland rice cultivation is source of soil erosion. This is especially true for hilly lands, such as those in Southeast Asia, where upland rice is grown in a traditional swidden system or under new plantings of tree crops such as rubber (Figure 4). Erosion of soil in the uplands fills downstream reservoirs with silt, thereby reducing the amount of water available for irrigated rice in the lowlands. Thus soil erosion has negative consequences on a regional level. The cultivation of perennial crops, such as perennial upland rice, would be expected to reduce soil erosion relative to current practices. This goal was the driving force behind the International Rice Research Institute (IRRI) Perennial Upland Rice Project during the 1990s. However, a successful cultivar of perennial upland rice would need exceptional drought tolerance to survive the long dry season that is typical in mainland SE Asia. Development of adapted perennial rice cultivars would be considerably less challenging for areas without a dry season or only a short dry season, than for long dry season environments. For example, in Mato Grosso, Brazil, some modern and high yielding cultivars of upland rice produce good ratoon crops under favourable environmental conditions (Santos *et al.* 2003).

FIGURE 4. UPLAND RICE PRODUCTION ON HILLY LANDS IN SOUTHEAST ASIA

Top: note upland rice on left side of photo among tree stumps from recently cleared land, and also note the large area of soil erosion on the steep area in the centre of the photo. Bottom left: upland rice growing under a new rubber plantation. Bottom right: a new rubber plantation with bare, eroding soil associated with a gap between the cycles of harvesting and planting of annual understory crops.

IMPROVING RATOONING ABILITY AND YIELD WITHIN *O. SATIVA*

Though ratoon cropping is practiced commercially in the southern United States of America and parts of southern China (Bollich and Turner, 1988; Xue-Bin *et al.* 1988), substantial improvements in regrowth ability and yield potential of ratoon crops would be needed for ratoon cropping to compete economically with new plantings from seed/seedlings in areas where conducive environmental conditions do not limit the time available for growing a subsequent rice crop. For those cultivars that can regrow well after harvest of an initial crop, first ratoon yields are typically 40 percent or less of main crop yields and subsequent decreases in yield make further cycles of ratooning currently uneconomical (Chauhan *et al.* 1985; Krishnamurthy, 1988). Thus, a key challenge is to breed cultivars that have high overall yield potential yet have high ratoon yields (at least on a per growing day basis) as well as high main crop yields. Though most ratoon crops have lower yields than main crops, there have been documented cases where both main crop and ratoon crop yields were both high and similar to each other, indicating that a possible path forward is to identify combinations of genotype and environment that result in multiple high yields. Another key challenge is to select genotypes that maintain high yields for more than one ratoon crop. Such a development would be a step-change for rice that would potentially make ratooning an economically attractive option for irrigated production environments that are not limited in duration by cold weather or other season-limiting conditions. High ratooning ability and low rates of missing hills are necessary but not sufficient for achieving high yields for one or more ratoon crops. Other desirable traits for improving ratoon cropping are drought tolerance, cold tolerance (especially at flowering stage for subtropical and temperate environments), duration adapted to the target environment, and highly resistant to pests and diseases.

Little information is available on the genetics of ratooning ability in rice. However, choice of parents can have a large effect, and heritabilities may be moderate to low (Shifen and Tingwen, 1988). Late stage selection is likely to result in slow and modest improvements. For greater and more rapid gains, early generation selection coupled with replication of genotypes would be desirable. Replicated early generation selection could be accomplished with doubled-haploid populations or by vegetatively propagating F_2 individuals.

INTROGRESSING GENES FOR PERENNIAL GROWTH FROM *O. RUFIPOGON* AND *O. LONGISTAMINATA*

Both *O. rufipogon* and *O. longistaminata* are sources of genes for improving perennation in *O. sativa*. Previous studies at IRRI indicated that choice of genotype within both the domesticated and undomesticated parent species has a large effect on the progenies' ability to grow perennially (Sacks *et al.* 2006, 2007). The main advantage of using *O. rufipogon* as a donor of genes for perennial growth to domesticated Asian rice is its high cross compatibility with *O. sativa*. However, stolons, which are the key perennating structure of *O. rufipogon*, are not well-suited to surviving drought because they may be exposed to sun and dry air on the surface of the soil if grown under upland conditions, or under rainfed production during the dry season. In contrast, the rhizomes of *O. longistaminata* are protected from desiccation by insulating soil. Additionally, *O. longistaminata*, which can form large monocultures in the wild, is more vigorous than *O. rufipogon*. Breeding barriers have typically limited the production of F_1 *O. sativa/O. longistaminata* progeny severely, and embryo rescue has usually been required to obtain early generation backcross progeny in large numbers (Chu and Oka, 1970; Hu *et al.* 2003; Ishikawa *et al.* 2011; Tao and Sripichitt, 2000). Moreover, associations between infertility and rhizomatous growth in early generations have required considerable breeding work to break (Chen *et al.* 2009). Recently however, Kanya *et al.* (2012) reported that crosses between *O. sativa* 'Basmati 370' and a Kenyan accession of *O. longistaminata* resulted in the production of over 500 hybrid seed (6 percent success) and that the F_1 hybrids, which germinated without embryo rescue, were remarkably fertile, producing more seed per plant than either parent. It is likely that *O. longistaminata* is the best species source of genes for improving regrowth potential of domesticated rice via introgression, given that it has vigorous perennial growth, great genetic diversity associated with broad geographic distribution and self-incompatibility, and the same A-genome as *O. sativa* (Kiambi *et al.* 2008; Melaku *et al.* 2013). The recent development of a whole genome fosmid library for *O. longistaminata* should facilitate identification and introgression of key genes from this species into domesticated rice (Li *et al.* 2012). Additionally, Shim (2012) has described several breeding strategies for introgressing genes from *O. longistaminata*. Though *O. longistaminata* is a promising source of genes for improving many traits in domesticated rice, few interspecific hybrids between *O. sativa* and *O. longistaminata* have yet been produced. A substantial public collection of *O. longistaminata* germplasm is available but it remains largely untapped.

In a hybrid population of *O. sativa* and *O. longistaminata*, rhizomatous growth was conferred by two dominant complementary genes for rhizome presence and many QTL of modest effect for degree of rhizome expression (Hu *et al.* 2003). Subsequently, candidate genes for rhizome expression were identified by gene expression analyses (Hu *et al.* 2011), which should further facilitate introgression efforts. Diligent breeding work over many years at the Yunnan Academy of Agricultural Sciences has resulted in the production of interspecifc progenies that have both long rhizomes and high fertility. Moreover, non-rhizomatous backcross progenies of *O. sativa/O. longistaminata* have been selected for their ability, under irrigated conditions, to produce three sequential harvests (one main crop and two ratoon crops) from a single planting, yet have high yields for all three harvests. Thus, introgression from *O. longistaminata* is also a useful strategy for improving ratooning in domesticated rice. Further use of *O. longistaminata* accessions and genes is expected to be valuable for rice improvement.

DOMESTICATING THE PERENNIAL RHIZOMATOUS SPECIES *O. LONGISTAMINATA* AND *O. AUSTRALIENSIS*

The main advantage of domestication as a breeding strategy over introgression is that breeding barriers associated with wide crosses are avoided. However, the main obstacle to domesticating a wild species is that desirable alleles for domestication and agronomic traits are in low frequency in the founder population. Thus, domestication can be expected to take considerable time and effort to produce acceptable cultivars for use by farmers. Nevertheless, domestication may be a viable option if breeding barriers will significantly limit introgression, and if many genes are needed from a wild species to achieve a particular breeding goal. For example, adaptation of rice to perennial growth while surviving seasonal drought may be a case in which domestication is the best option. Both *O. longistaminata* and *O. australiensis* have rhizomes which may enable the plants to survive in a dormant state during drought (Henry *et al.* 2010). Though this dormancy response has not been thoroughly explored, we expect that the trait is most strongly expressed in *O. australiensis* because its native habitat is more drought prone than that of *O. longistaminata*. Introgression may be a competitive option for developing drought-resistant perennial upland rice from *O. longistaminata*, but for the E-genome *O. australiensis*, severe breeding barriers to crossing with *O. sativa* would make domestication the only practical option. With their long, vigorous rhizomes and shattering seed, both *O. longistaminata* and *O. australiensis* are potentially invasive, which would be of particular concern outside of their native range. Thus, any domestication efforts would need to select for a moderate rhizome length, which balances the need for survival during drought with the need to minimize potential invasiveness. For initial breeding work to domesticate *O. longistaminata* or *O. australiensis*, a location where the species are native would be most desirable, so as to avoid introducing potentially invasive early generation plants to areas where they are non-native. In addition to reduced rhizome length, domesticated versions of these species would need to be non-shattering, semi-dwarf, and high-yielding. Fortunately, the sequence of many key genes for domestication of *O. sativa*, such as the non-shattering genes *sh4* and *qSH1*, and the semi-dwarf gene *sd1* are known (Konishi *et al.* 2006; Li *et al.* 2006; Monna *et al.* 2002), which should allow for relatively rapid domestication of wild rice species via targeted screening of germplasm and selection.

REFERENCES

Bollich, C.N. & Turner, F.T. 1988. Commercial ratoon rice production in Texas, USA. In: *Rice Ratooning*. IRRI, Los Baños, Philippines, pp. 257-263.

Cheng, C.Y., Motohashi, R., Tsuchimoto, S., Fukuta, Y., Ohtsubo, H. & Ohtsubo, E. 2003. Polyphyletic origin of cultivated rice: based on the interspersion pattern of SINEs. *Molecular Biology and Evolution*. 20: 67–75.

Chauhan, J.S., Vergara, B.S. & Lopez, F.S. 1985. Rice Ratooning. IRRI, *Research Paper Series*. 102–19.

Chen, Z., Hu, F., Xu, P., Li, J., Deng, X., Zhou, J., Li, F., Chen, S. & Tao, D. 2009. QTL analysis for hybrid sterility and plant height in interspecific populations derived from a wild rice relative, *Oryza longistaminata*. *Breeding Science*. 59: 441–445.

Chu, Y.E. & Oka, H.I. 1970. The genetic basis of crossing barriers between *Oryza perennis* subsp. *barthii* and its related taxa. *Evolution*. 24: 135–144.

de la Rosa, J.S. 2004. Harvest more rice with ratooning. *BAR Digest*. 6: 4.

Henry, R.J., Rice, N., Waters, D.L.E., Kasem, S., Ishikawa, R., Hao, Y., Dillon, S., Crayn, D., Wing, R. & Vaughan, D. 2010. Australian *Oryza*: Utility and conservation. *Rice*. 3: 235–241.

Hill, R.D. 2010.The cultivation of perennial rice, an early phase in Southeast Asian agriculture? *Journal of Historical Geography*. 36: 215–223.

Hu, F.Y., Tao, D.Y., Sacks, E., Fu, B.Y., Xu, P., Li, J., Yang, Y., McNally, K., Khush, G.S., Paterson, A.H. & Li, Z-K. 2003. Convergent evolution of perenniality in rice and sorghum. *Proceedings of the National Academy of Sciences USA*. 100: 4050-4054.

Hu, F., Wang, D., Zhao, X., Zhang, T., Sun, H., Zhu, L., Zhang, F., Li, L., Li, Q., Tao, D., Fu, B. & Li, Z. 2011. Identification of rhizome-specific genes by genome-wide differential expression Analysis in *Oryza longistaminata*. *BMC Plant Biology*. 11: 1471-2229.

Ishikawa, R., Ohnishi, T., Kinoshita, Y., Eiguchi, M., Kurata, N. & Kinoshita, T. 2011. Rice interspecies hybrids show precocious or delayed developmental transitions in the endosperm without change to the rate of syncytial nuclear division. *The Plant Journal*. 65: 798–806.

Kanya, J.I., Hauser, T.P., Kinyamario, J.I., Amugune, N.O. 2012. Hybridization potential between cultivated rice *Oryza sativa* and African wild rice *Oryza longistaminata*. *International Journal of Agricultural Research*. 7: 291-302.

Kiambi, D.K., Newbury, H.J., Maxted, N. & Ford-Lloyd, B.V. 2008. Molecular genetic variation in the African wild rice *Oryza longistaminata* A. Chev. et Roehr. and its association with environmental variables. *African Journal of Biotechnology*. 7: 1446-1460.

Konishi, S., Izawa, T., Lin, S.Y., Ebana, K., Fukuta, Y., Sasaki, T. & Yano, M. 2006. An SNP caused loss of seed shattering during rice domestication. *Science*. 312: 1392-1396.

Krishnamurthy, K. 1988. Rice ratooning as an alternative to double cropping in tropical Asia. In: *Rice Ratooning*. IRRI, Los Baños, Philippines, pp. 3-15.

Li, L., Cai, J., Zhang, S., Wang, L. & Hu, F. 2012. Construction of whole genome fosmid library in Africa wild rice (*Oryza longistaminata*). *Rice Genomics and Genetics*. 3: 66-71.

Li, C., Zhou, A. & Sang, T. 2006. Rice domestication by reducing shattering. *Science*. 311: 1936-1939.

Lu, H., Redus, M.A., Coburn, J.R., Rutger, J.N., McCouch, S.R. & Tai, T.H. 2004. Population structure and breeding patterns of 145 USA rice cultivars based on SSR marker analysis. *Crop Science*. 45: 66–76.

Melaku, G., Haileselassie, T., Feyissa, T. & Kiboi, S. 2013. Genetic diversity of the African wild rice (*Oryza longistaminata* Chev. et Roehr) from Ethiopia as revealed by SSR markers. *Genetic Resources and Crop Evolution*. 60: 1047–1056.

Monna, L., Kitazawa, N., Yoshino, R., Suzuki, J., Masuda, H., Maehara, Y., Tanji, M., Sato, M., Nasu, S. & Minobe, Y. 2002. Positional cloning of rice semidwarfing gene, sd-1: rice “green revolution gene” encodes a mutant enzyme involved in gibberellin synthesis. *DNA Research.* 9: 11–17.

Munda, G.C., Das, A. & Patel, D.P. 2009. Evaluation of transplanted and ratoon crop for double cropping of rice (*Oryza sativa* L.) under organic input management in mid altitude sub-tropical Meghalaya. *Current Science.* 96: 1620-1627.

Sacks, E.J., Dhanapala, M.P., Sta. Cruz, M.T. & Sallan, R. 2006. Breeding for perennial growth and fertility in an *Oryza sativa/O. longistaminata* population. *Field Crops Research.* 95: 39–48.

Sacks, E.J., Dhanapala, M.P., Sta Cruz, M.T. & Sallan R. 2007. Clonal performance of perennial *Oryza sativa/O. rufipogon* selections and their combining ability with *O. sativa* cultivars for survival, stolon production and yield. *Field Crops Research.* 100: 155-167.

Sanni, K.A., Ojo, D.K., Adebisi, M.A., Somado, E.A., Ariyo, O.J., Sie, M., Akintayo, I., Tia, D.D., Ogunbayo, S.A., Cisse, B., Sikirou, M. & Adekoya, M.A. 2009. Ratooning potential of interspecific NERICA rice varieties (*Oryza glaberrima* × *Oryza sativa*). *International Journal Botany.* 5: 112-115.

Santos, A.B., Fageria, N.K. & Prabhu, A.S. 2003. Rice ratooning management practices for higher yields. *Communications in soil science and plant analysis.* 34: 881–918.

Shahi, B.B., Jeanine. & Raharinirian, J. 1988. Potential of rice ratooning in Madagascar. In: *Rice Ratooning.* IRRI, Los Baños, Philippines, pp. 129-134.

Shifen, L. & Tingwen, C. 1988. Inheritance of ratooning ability in Chinese rice varieties[*O. sativa* subsp. *xian* (or indica)]. In: *Rice Ratooning.* IRRI, Los Baños, Philippines, pp. 239-245.

Shim, J. 2012. Perennial rice: Improving rice productivity for a sustainable upland ecosystem. *SABRAO Journal of Breeding and Genetics.* 44: 191-201.

Tao, D. & Sripichitt, P. 2000. Preliminary report on transfer traits of vegetative propagation from wild rice species to *Oryza sativa* via distant hybridization and embryo rescue. *Kasetsart Journal Natural Science.* 34: 1-11.

Xue-Bin, X., Jong-Guo, Z. & Xian-Xiang, J. 1988. Ratooning in China. In: *Rice Ratooning*. IRRI, Los Baños, Philippines, pp. 79-85.

03

THE PROGRESSION OF PERENNIAL RICE BREEDING AND GENETICS RESEARCH IN CHINA

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ABSTRACT

Soil erosion is a worldwide problem of increasing concern, and perennial grain crops could be an important part of the solution. In Southeast Asia, upland rice (*O. sativa*) contributes to regional soil erosion problems because it is an annual crop grown on hilly lands. The perennial cultivars of upland rice could reduce soil erosion and meet the needs of subsistence farmers. From the viewpoint of breeding, *O. longistaminata*, with same genome, AA, similar to *O. sativa*, would be the most logical donor of genes for rhizome expression for perennial rice cultivar development,

several donor traits, such as rhizome and stolon have been employed for perenniality. Up to now, there are some results as following: 1) based on the fine mapping of the rhizome genes (*Rhz*), via genomic library (BAC, Fosmid, rhizome cDNA library) construction and analysis, confirming the genetic regularity that the rhizome was controlled by two pairs of dominant complementary genes, *Rhz2*、*Rhz3*, and obtaining 15 rhizome locus and candidate functional genes; 2) the perennial rice breeding is on the way and some breeding lines that hold the rhizome genes were made. There are five perennial rice (PR) lines, namely PR23, PR57, PR129, PR137 and PR139, that have been bred; 3) the potential perennial materials were screened at different sites, such as Lao, Africa and China for perennial ability investigation. Thus development of perennial rice cultivars from *O. longistaminata* faces two large challenges: 1) the need to pyramid in an *O. sativa* background multiple rhizome QTL in order to get strong rhizome expression, and 2) the need to get rid of QTLs for low pollen fertility without losing linked QTLs for rhizomes.

Keywords: perennial rice, breeding, genetics research, *Oryza longistaminata*

INTRODUCTION

Soil erosion is a serious problem in the uplands of Southeast Asia (Figure 1). Once forest is cleared on sloping uplands and replaced with annual crops such as upland rice, nutrients are rapidly leached and soils are eroded, so lands need to return to forest for some years before being suitable again for crop production. Population pressure is reducing the duration of fallow, so performance of upland rice, associated crops and livestock are declining, as soil quality and fertility progressively deteriorate. A way is needed to stabilise these fragile soils (IRRI, 1998).

FIGURE 1. SOIL EROSION IN UPLAND RICE FIELD IN YUNNAN, CHINA

Soil erosion in uplands of southeast Asia has been a serious problem that led to the project of developing perennial upland rice at IRRI (IRRI 1989)

TABLE 1. *ORYZA* SPECIES, THE SPECIES COMPLEX, CHROM., GENOME GROUP AND DISTRIBUTION

SECTION	COMPLEX	SPECIES	CHROMOSOME NUMBER	GENOME GROUP	DISTRIBUTION
ORYZA	*O. sativa* complex	*O. sativa* L.	24	AA	worldwide
		O. nivara Sharma et Shastry	24	AA	Tropical and Sub. Asia
		O. rufipogon Griff	24	AA	Tropical and Sub. Asia
		O. meridionalis Ng	24	A^mA^m	Tropical and Australia
		O. glumaepatula Steud.	24	$A^{gl}A^{gl}$	South America
		O. glaberrima Steud	24	A^gA^g	Africa (mainly West)
		O. barthii A. Chev.	24	A^gA^g	Africa
		O. longistaminata Chev. et Roher	24	A^lA^l	Africa
	O. officinalis complex	*O. officinalis* Wall ex Watt	24	CC	Tropical and Sub. Asia
		O. minuta Presl. et Presl.	48	BBCC	Philippines
		O. eichingeri Peter	24	CC	Sri Lanka, Africa
		O. rhizomatis Vaughan	24	CC	Sri Lanka
		O. punctata Kotschy ex Steud.	24, 48	BB, BBCC	Africa
		O. latifolia Desv.	48	CCDD	Latin America
		O. alta Swallen	48	CCDD	Latin America
		O. grandiglumis (Doell) Prod.	48	CCDD	South America
		O. australiensis Domin	24	EE	Australia
RIDLEYANAE TATEOKA		*O. brachyantha* Chev. et Roehr.	24	FF	Africa
		O. schlechteri Pilger	48	HHKK	Papua New Guinea
	O. ridleyi complex	*O. ridleyi* Hook. f.	48	HHJJ	SE Asia
		O. longiglumis Jansen	48	HHJJ	Irian Jaya, Indonesia
GRANULATA ROSCHEV.	O. meyeriana complex	*O. meyeriana* Baill	24	GG	SE Asia
		O. granulata Nees et Arn. ex Watt	24	GG	S and SE Asia

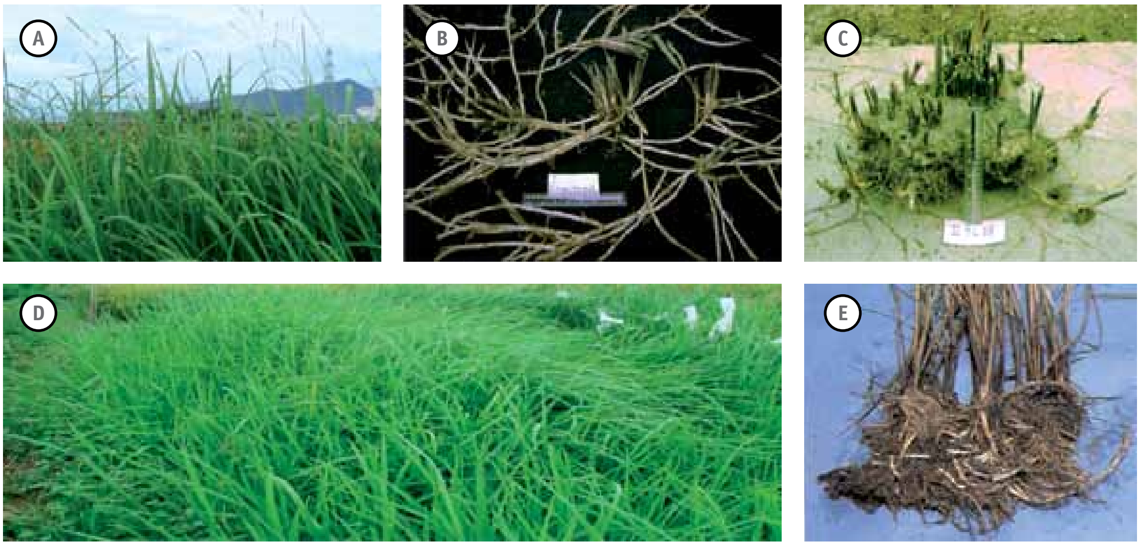
Following reports of a successful cross between *Oryza sativa* and *O. longistaminata* at the Yunnan Academy of Agricultural Sciences (Tao, 2000), development of perennial rice was proposed as one way to maintain surface cover after clearing, with potential benefits to nutrient and soil retention, and rice and livestock performance (IRRI, 1998). With donor support from Europe, research commenced at IRRI, with several reports explaining the concept, outlining the approach and reporting some initial results (Xiu, 1995; Schmit, 1996; Tao, 2000; Sacks, 2003).

METHODS

The *Oryza* species are quite diverse, as indicated in Table 1, which lists the species with their chromosome number, genome group and distribution by *Oryza* complex. The species arrowed express perenniality, including *O. longistaminata* in the *O. sativa* complex, and *O. rhizomatis* and *O. australiensis* in the *O. officianalis* complex.

Initially, *O. sativa* was crossed with *O. longistaminata* and *O. rufipogon*, but the crosses were more successful with *O. longistaminata*, as it is in the same complex as *O. sativa*. Consequently, it was decided to use *O. longistaminata* predominantly as the source of perenniality. *O. longistaminata* is AA genome like *O. sativa*, and develops extensive rhizomes in its native wetland habitat (Figure 2).

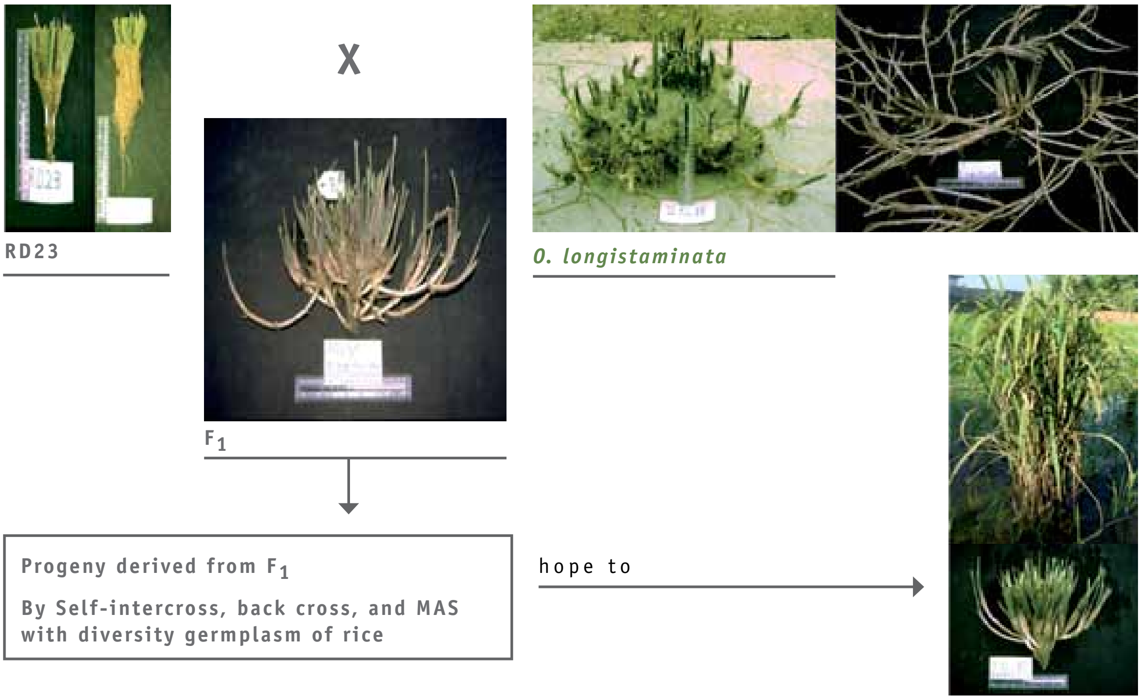
FIGURE 2. THE *O. LONGISTAMINATA* (AA GENOME)



The *Oryza longistaminata*
A. The panicle of the *O. longistaminata*
B,C,E. The strong rhizomes of *O. longistaminata*
D. The performance of *O. longistaminata* in field

The breeding strategy adopted to develop perennial rice at Yunnan Academy of Agricultural Sciences was as follows. RD23 was chosen as the *O. sativa* parent, as this cultivar was widely grown in lowland or upland, high yielding, good grain quality glutenous, and with disease resistance to rust etc. It was crossed with *O. longistaminata*, and the F_1 was intermediate in characteristics between the parents (Figure 3).

FIGURE 3. THE STRATEGY FOR PERENNIAL RICE BREEDING



From a combination of intercrossing among the F_1 progeny, backcrossing to RD23, and selection for desired traits, progress was made in developing perennial rice (Figure 3). This process was repeated a number of times using a wide range of successful cultivars as the *O. sativa* parent, but to date, the most successful has been with RD23. Selection, including marker-aided selection (MAS), was used to assist in transferring the perenniality traits from *O. longistaminata* into perennial rice. This followed successful research to identify QTL associated with rhizome development in *O. longistaminata*.

RESULTS

1. Genetic analysis of rhizome production

Genetics of rhizome expression was explored with Simple-Sequence Repeats (SSR) markers on the F_2 population from RD23/ *O. longistaminata*, using field and marker data. The PCR-based molecular genetic map (Figure 4) revealed three regions on chromosomes 3 and chromosome 4 of rice that indicated the two dominant complementary genes for rhizome expression, which were designated as *Rhz2* and *Rhz3*, respectively (Hu, 2001; Hu, 2003).

FIGURE 4. MOLECULAR GENETIC MAP OF RD23_LONGI BY SSR MARKERS

PCR-BASE MOLECULAR GENETIC MAP

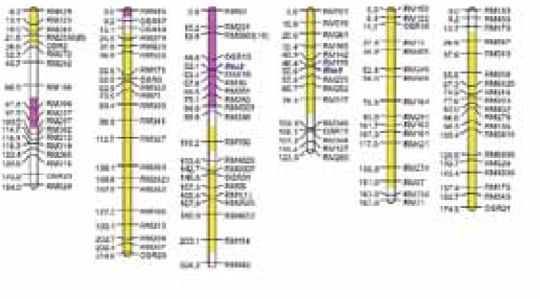
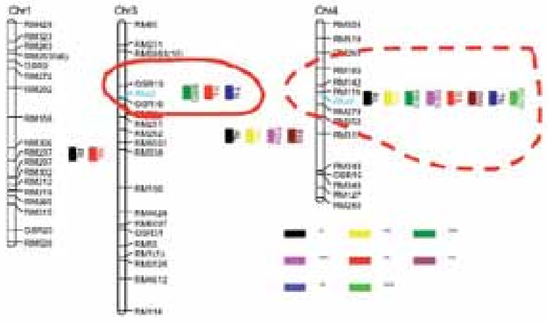


FIGURE 5. QTLs ANALYSIS OF RHIZOME RELATED TRAITS

THE QTLS OF RHIZOME TRAITS MAPPING ON CHROMOSOME



Rhz3 on chromosome 4 co-located with many related root traits, including root length, root number, root branching density, root branching number, root internode length, root internode number, tiller number and root dry weight (Figure 5). Likewise, *Rhz2* on Chromosome 3 was co-located with root branching density, root internode length and tiller number, with the other traits closely located on the same arm of chromosome 3. The strong association between rhizome QTLs and related root QTLs lends confidence, as rhizome formation should lead to changes in the other parameters (Hu, 2003).

Subsequent fine mapping for *Rhz3* identified flanking markers RM14603 and OSR16 about 35 kb apart for *Rhz2* in 2008, and in 2012, a functional 5 scaffold was determined (Figure 6). Likewise, for Rhz3, the flanking markers were RM119 and RM17000 at 9.528 kb apart, with a 6 scaffold determined (Figure 7).

FIGURE 6. THE *RHZ2* FINE MAPPING

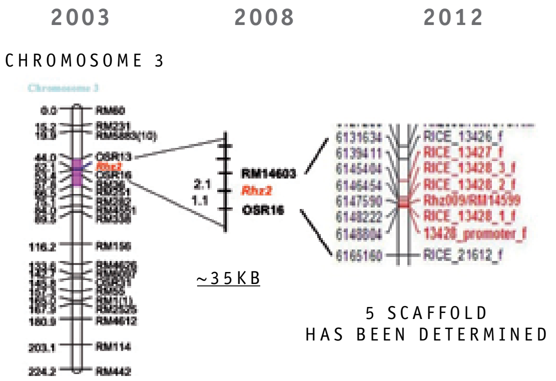
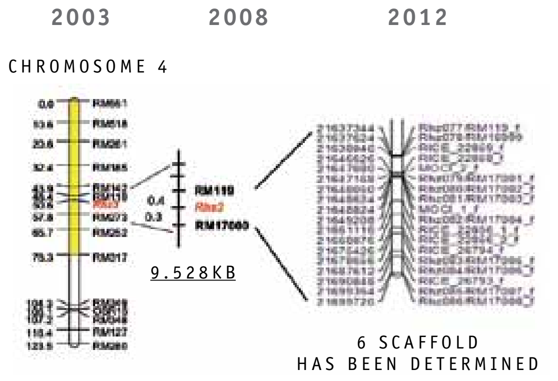


FIGURE 7. THE *RHZ3* FINE MAPPING



2. Breeding of perennial rice

Materials were initially evaluated and selected in the greenhouse, before being evaluated and selected in several field environments. Sanya on Hainan Island in the south of China was used from 2007 to 2010 to allow rapid generation advance under tropical lowland paddy conditions, as two crops per year were possible there. From 2011, however, the breeding station was moved from Sanya to Jing Hong in southern Yunnan Province, with additional field sites established on different soils at Puer and Menglien, which were perceived to be more appropriate environments for perennial rice.

Field evaluation demonstrated that plants were able to regrow successfully in the field for at least three seasons under these conditions, demonstrating not only regrowth and survival, but also reproductive success and grain set in the field.

The outstanding line was PR23 derived from RD23/ *O. longistaminata*, although PR57, PR129 and PR137 were also promising. Field performance of PR23 is after grain harvest in Simao, during regrowth in Simao and Jing Hong, and as a mature crop close to harvest in its third year in Jing Hong (Figure 8). A close-up of PR23 regrowth relative to senesced stems cut in the previous year is shown (Figure 9), along with a close-up of dry season survival at Puer (Figure 10).

FIGURE 8. THE PR LINES: PR23

The phenotype of PR23 in Jinghong, 2012/5 during Len Wade visiting there

FIGURE 9. THE REPRODUCTION ABILITY OF PR23

RE-GROWTH FROM UNDERGROUND

THE OLD STEMS

The growth situation of PR23 after cutting the stub back to 10-15 cm 2 months in Jinghong (The third growth season)

FIGURE 10. THE REPRODUCTION ABILITY OF PR23

PR23 leave in field for next season growth 2011/12, Simao, Yunnan

TABLE 2. YIELD VARIATION OF PR23 BETWEEN DIFFERENT GROWTH SEASONS AND SITES

CROP SEASON	PHENOLOGICAL STAGE	YIELD OF PER UNIT AREA (KG/HA)	DEATH RATE (OVER YEAR)	SITE
First growth season	2011/1/10-2011/6/10 150d	5619.83		Jinghong, Yunnan, China
Second growth season	2011/6/13-2011/10/22 131d	3905.05		
Third growth season	2012/2/10-2012/6/5 116d	4027.57[a]	3.5%	
First growth season	2011/3/7-2011/8/10 157d	7350.00		Simao, Yunnan, China
Second growth season	2011/8/30-	The temperature was too low that the grain setting rate was not very good		
Third growth season	2012/3/28-2012/8/15 137d	6720.00	5.4%	

The yield performance of PR23 perennial rice over three seasons in the field at Jing Hong and Simao is shown in Table 2. Yields from the first wet season were impressive at 5.6 and 7.4 tonnes/ha. Not surprisingly, yields were lower in the drier second season when temperatures were lower in winter. Survival in the second wet season was impressive, with about 5 percent loss in plant stand over the first year. Nevertheless, wet season yields in the second year declined to about 70 and 90 percent of yields in the first wet season, respectively, which may reflect different seasonal conditions, some decline in plant vigour, or both. What factors may contribute to such changes needs further investigation, even though yields were still impressive in season three at 4.0 tonnes/ha (with some rat damage) and 6.7 tonnes/ha.

As a result of the strong performance of PR23 in the field, and farmer and district interest at the field sites, PR23 has entered pre-release testing for release as a perennial rice cultivar in Yunnan Province. At this stage, an individual farmer can try a small area, but a further three years of field evaluation data at several sites in Yunnan is needed before formal release of the cultivar, and any approval to increase and release seed to farmers. Nevertheless, the entry of PR23 to pre-release testing in Yunnan is a first for the perennial crops community. While some perennial species have been improved, and some fortuitous discoveries of unknown origin have been identified and grown by a few farmers, this is the first report of the intentional selection of a perennial cultivar from a cross with a related wild perennial species. This represents a milestone in combining a capacity to regrow with a capacity to set grain in subsequent generations. Hopefully, this heralds the advent of further breeding success in the perennial grains community.

3. Collaborations in perennial rice

In 2011, collaboration was established in neighbouring Lao, PDR via the ACIAR project on developing improved farming and marketing systems for rainfed regions of southern Lao PDR (Wade and Sengxua, 2014). A set of 13 recombinant inbred lines from the cross RD23/ *O. longistaminata* was planted at Xepon in Savannakhet Province and Phone Ngam in Champassak province in southern Lao PDR (Figure 11). The lines performed well in the first wet season (Table 3), but there was some stand loss in the harsher conditions encountered on light-textured soils in southern Lao, PDR, despite life-saving applications of water during the dry season. Some plants of most entries did regrow at both sites in the second wet season, but at one site, the farmer allowed livestock to graze at break of wet season, and heavy rain and flooding shortly thereafter resulted in total crop loss at Xepon. At the other site, the crop survived the typhoon, but plant vigour was affected and greater weed competition resulted, so yields at Phone Ngam were much lower in the second wet season (Table 3). A second set of 22 recombinant lines has now been sent to Lao, PDR for evaluation, and with the experience provided by this initial attempt, should be conducted with better management next time. Nevertheless, the capacity to regrow and produce forage and grain in the second year was recognised by the collaborators, who wish to continue this research in perennial rice. The plant breeders in particular were keen to collaborate in this research.

FIGURE 11. PERENNIAL RICE LINES GROWN IN XEPON, SAVANNAKHET AND PHONENGAM, PAKXE, CHAMPASAK, FOR ONE (2011) AND TWO (2011-2012) YEARS, RESPECTIVELY

TABLE 3. ANALYSIS OF VARIANCE OF GRAIN YIELD (g/m^2) OF 13 PERENNIAL RICE LINES GROWN IN XEPON, SAVANNAKHET AND PHONENGAM, PAKXE, CHAMPASAK, FOR ONE (2011) AND TWO (2011-2012) YEARS, RESPECTIVELY

SOURCE	df	TYPE II SS	MS	F	P
MAIN EFFECTS					
Year	1	1172206.515	1172206.5	1138.8705	.0000***
Site	1	413.1565707	413.15657	0.4014069	.5283 ns
Entry	12	140679.6121	11723.301	11.389905	.0000***
INTERACTION					
Year x Entry	11	39082.49769	3552.9543	3.4519128	.0007***
Site x Entry	11	30768.72232	2797.1566	2.717609	.0053**
Error	74	76166.06598	1029.2712<-		
Total	110	1893016.645			
Model	26	1816850.579	50468.072	49.032824	.0000***

R^2 = SSmodel/SStotal = 0.95976471407

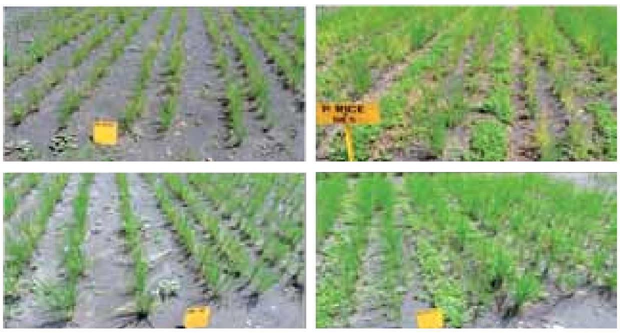
Root MSerror = sqrt (MSerror) = 32.0822561844

Mean Y = 188.996693703

Coefficient of Variation = (Root MSerror)/ abs (Mean Y) * 100% = 16.975036%

A related evaluation conducted in Nairobi, Kenya has confirmed the initial set of materials could not cope well with dry periods and their associated water deficits (Figure 12). Further research is needed to develop perennial rices able to cope with the severity of abiotic stresses under rainfed conditions, including drought in rainfed lowland and especially rainfed uplands. An optimistic note was provided by plant survival after three seasons on rainfall alone at Puer. The soil there is heavier with greater water-holding capacity, and temperatures in the dry season are cooler at higher elevation, so water loss is reduced, and plants survived. This observation lends confidence to the prospect for further improvements in dry season survival.

FIGURE 12. THE PERENNIAL ABILITY TEST OF PR23 IN AFRICA



DES: DESMODIUM
did not respond well to extreme drought conditions

CONCLUSIONS

A successful perennial rice breeding program has been established at Yunnan Academy of Agricultural Sciences, with the line PR23 now in pre-release testing in Yunnan Province. This success is based on a strategy of intercrossing F_1 lines, backcrossing to the cultivated rice parent, and rigorous selection for survival and seed set in the field. Development of perennial rice is consequently at the forefront of perennial grain development, and will hopefully act as an incentive to success in other species. The time is ripe to build on this success by establishing a consortium of perennial crop researchers, supported by a suite of donors to ensure the continuity of efforts needed for success in this challenging but important endeavour. Despite some success in developing a perennial rice phenotype which may be suitable for more favourable lowland conditions in which abiotic stresses are minimal, significant challenges remain in developing a robust perennial rice for the harsher rainfed lowland and especially upland ecosystem, where perennial rice is really needed.

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REFERENCES

Hu, F.Y., Xu, P., Yang, Y., McNally, K., Zhou, J., Tao, D.Y., Li, J., Sacks, E., Cruz, T.S. & Li, Z.K. 2001. Two dominant complementary genes controlling rhizomatous expression in *Oryza longistaminata*. *Rice Genetics Newsletter.* 18: 34-36.

Hu, F.Y., Sacks, E., Fu, B.Y., Xu, P., Li, J., Yang, Y., McNally, K., Khush, G.S., Paterson, A.H. & Li, Z-K. 2003. Convergent evolution of perenniality in rice and sorghum. *Proceedings from the National Academy of Science*. *USA*. 100(7): 4050-4054.

International Rice Research Institute. 1998. *IRRI toward 2000 and beyond*. IRRI, Manila. pp. 66.

Tao, D., Hu, F.Y., Yang, Y., Xu, P., Li, J., Wen, G., Sacks, E., McNally, K. & Sripichitt, P. 2000. Preliminary report on transfer of vegetative propagation from wild rice to *O.* sativa via distant hybridization and embryo rescue. *Kasetsart Journal of Natural Science*. 34: 1-11.

Schmit, V. 1996. Improving sustainability in the uplands through the development of a perennial upland rice. In: Piggin, C., Courtois, B. & Schmit, V. (eds.), Upland rice research in partnership. *Proceedings of the upland rice consortium workshop*. January 1-13, 1996, Padang, Indonesia. Manila, International Rice Research Institute, p 265-273.

Sacks E.J., Roxas, J.P. & Cruz, M.T.S. 2003. Developing perennial upland rice II: Field performance of S1 families from an intermated *Oryza sativa/ O. longistaminata* population. *Crop Science*. 43: 129-134.

Wade, L.J. & Sengxua, P. 2014. Developing improved farming and marketing systems in rainfed regions of southern Lao PDR: Synthesis of the Southern Lao Project. *Lao Journal of Agriculture and Forestry*. (In press).

Xiu L.Q. 1995. Breeding for perennial hybrid rice. *Division Seminars of Plant Breeding, Genetics, and Biochemistry*, IRRI, Manila, Philippines.

04

PERENNIAL WHEAT BREEDING: CURRENT GERMPLASM AND A WAY FORWARD FOR BREEDING AND GLOBAL COOPERATION

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ABSTRACT

The Australian perennial wheat team has collected and assessed a wide range of global germplasm derived from wheat x perennial-relative crosses (Hayes *et al.* 2012 and unpublished). Some lines were able to perenniate in one or both field environments where they were grown and a few were able to regrow through four seasons. Without exception the only wheat-derived lines that could perenniate contained seven or more pairs of chromosomes from the perennial parent. The donor perennial parents included *Thinopyrum ponticum* (2n = 70, decaploid), *Th. intermedium* (2n = 42, hexaploid) and *Th. elongatum* (2n = 14, diploid). This has led us to conclude that the best near-term prospect for a perennial wheat-like grain crop is a full or partial amphiploid, containing the full set of tetraploid (AABB) or hexaploid (AABBDD) wheat chromosomes plus one genome (XX) from the donor. When the perennial donor is a polyploidy, the extra genome is usually a synthetic genome, consisting of a mixture of chromosomes from the polyploid parental genomes. This creates a significant difficulty in that each time a primary partial amphiploid is produced, the synthetic genome may consist of a different mix of donor chromosomes, and therefore interbreeding of these primary partial amphiploids may result in poor fertility and loss of the perennial donor chromosomes. That, in turn, would be problematic in trying to establish a breeding program. Consequently we would advocate a breeding program based on a diploid perennial donor, such as *Th. elongatum* (EE). This is analogous to triticale breeding. The amphiploids produced could be AABBEE (analogous to hexaploid triticale, AABBRR) or AABBDDEE (analogous to octoploid triticale, AABBDDRR). We would further advocate a multinational effort to produce many primary amphiploids using locally adapted wheat parents and diverse accessions of *Th. elongatum*; the primary amphiploids would be shared among participating groups, and intercrossed to permit subsequent selection of robust, productive, locally adapted perennials. The analogy with triticale is instructive also in suggesting that many generations of selection may be required before full fertility, high yielding types can be recovered.

Keywords: amphiploidy, genomic changes, perennial wheat, rye, *Thinopyrum*, triticale

INTRODUCTION

This paper explores the question of the preferred breeding route to a successful wheat-like and wheat-derived perennial cereal. Other papers in the Workshop will explore related aspects such as the physiology and field performance of available perennial wheat germplasm and how perennial wheat might usefully fit into a mixed farming situation. Some of that information is assumed as a starting point for this consideration of the best genetic configuration for a perennial wheat and how to instigate a breeding program to move us from basic proof of principle to genotypes that can be successfully and usefully deployed into real farming systems.

LIKELIHOOD OF A PERENNIAL TRUE WHEAT

Our studies of the available germplasm have established the essential feasibility of the concept of a perennial wheat (Hayes *et al.* 2012 and unpublished). Lines were found that could regrow after grain harvest for up to four seasons; simulated grazing of several lines showed an ability to produce both valuable forage and grain; and in addition we could demonstrate their ability to accumulate much greater root biomass after the first season as an indicator of the environmental benefit that could accrue (Larkin *et al.* 2014). The grain quality of these lines even had potential for bread making, and we along with others have also demonstrated the disease resistances that had been contributed from the donors of perenniality (Cox *et al.* 2002; 2005; Murphy *et al.* 2009; Hayes *et al.* 2012). Despite this promise, few of the lines were developed from adapted wheat species and their grain yields were generally low and declining in subsequent years. The available germplasm does not meet the standard required for robustness of the perennial habit or the consistency of grain yield from one season to the next. What we concluded for the Australian environment, Murphy *et al.*(2010), Cox *et al.*(2010) and Jaikumar *et al.*(2012) concluded for North America, namely that better adapted and more productive germplasm is required. For decades perennial wheat breeding attempts in the United States and the former USSR held some hope that it might be possible to introgress the controlling genes for the perennial habit into the wheat genomes through recombination or chromosomal translocation. Indeed Lammer *et al.* (2004) gave impetus to this hope when they showed some capacity to regrow in the wheat cv. Chinese Spring, carrying an extra pair of 4E chromosomes from *Thinopyrum elongatum*, a perennial *Triticeae* species. However, the ability of this chromosome addition line to regrow and set seed a second time was not as strong as the perennial amphiploid progenitor, which itself was not very robust. Perenniality appears to be a polygenic trait (Zhao *et al.* 2012), and it will not be readily conferred by simple introgression from a perennial to an annual species. Indeed the likely genetic complexity of the perennial habit suggests the possibility that it might be easier to transfer domestication traits to an existing perennial *Triticeae* species. These traits would include non-shattering heads, free-threshing grain, more determinate flowering and larger seed size. The genetic controls for some domestication traits are relatively simple (Faris *et al.* 2003; Sang *et al.* 2009; Gegas *et al.* 2010; Takahashi and Shimamoto, 2011; Peng *et al.* 2011). Others such as threshability may be multigenic (Peleg *et al.* 2011).

PERENNIAL GRAIN BY DOMESTICATION

In Australia some herbaceous native legumes have been investigated as having potential as perennial grain crops through a process of domestication (Bell *et al.* 2012). Attempts have been made to commercialize grasses such as *Microlaena stipodies* and *Distichlis palmeri* as perennial grain crops (O'Neill, 2007; Kasema *et al.* 2010; Pearlstein *et al.* 2012). However, this has met with limited success. For the Australian native rice-related perennial grass *M. stipoides,* the major missing traits included synchronous maturity and resistance to shattering (Davies *et al.* 2005). The seeds are structurally similar to rice and some ecotypes have seeds almost as large as domesticated rice. Malory *et al.* (2011) characterized 18 genes from *Microlaena* which are homologues of rice genes known to be important in domestication. If successful, domestication of wild perennial grains will produce crops, which are only likely to have value for niche markets in the medium term. Lower flour yields and higher bran and fibre content per kernel are often an impediment to acceptance in standard milling markets for new domesticated grains (Bell *et al.* 2010). We will hear more from others at this meeting on the prospects for domesticating perennial species.

The Land Institute is also making good progress in direct domestication of a number of perennial species including the wheat relative *Thinopyrum intermedium* (kernza) (DeHaan *et al.* 2005; Van Tassel *et al.* 2010; Culman *et al.* 2013). What might be needed to advance success more rapidly down this domestication option is to utilize the expanding understanding of domestication genes in a more targeted way. Transgenic technology offers a powerful way to test the utility of specific genes in achieving domestication traits. Thereafter, if the course to commercial release is deemed too difficult for the transgenic plants, the same traits might be pursued through targeted mutations using technology such as TILLING or one of the emerging methods for site-directed genome modifications (Curtin *et al.* 2012). Genome editing technology employs sequence-specific nucleases to generate site-specific double-stranded Deoxyribonucleic Acid (DNA) breaks. Nuclease systems in development include: zinc finger nucleases; transcription activator-like effector nucleases (TALENs); and LAGLIDADG homing endonucleases (Curtin *et al.* 2012). The site-specific double strand DNA breaks are followed by informed and designed mutations, sequence insertions or replacements. Rapidly evolving genomic and molecular genetic technology may make rapid domestication of existing well-adapted perennials feasible, but not without substantial investment in the various species of interest to this Workshop and for the goal of a perennial grain crop.

PERENNIAL CEREAL GRAIN THROUGH HYBRIDISATION AND ALLOPOLYPLOID FORMATION

Hybridisation between annual grain crops and perennial relatives offers an avenue to combine the traits of perenniality and grain quality in a new crop species. We believe this would reduce the time of development, compared with domestication, and offer a product that might directly replace annual grains in the market place. There have been attempts to achieve this historically in Russia and the United States (Tsitsin and Lubimova, 1959; Suneson *et al.* 1964). Experimental breeding lines of hybrid perennial cereals have shown higher mineral and protein content than annual cultivars (Murphy *et al.* 2009) and some have demonstrated useful flour, dough and baking properties (Hayes *et al.* 2012). Perennial grains will need to be profitable if they are to be adopted widely in agriculture (Bell *et al.* 2008; Reeling *et al.* 2012) and direct access to already established markets will be important for the acceptance of perennial grains.

The rest of this paper focuses on wide hybridisation to combine perenniality with already domesticated wheat. Our experience thus far consistently indicates that reasonable rates of post-harvest regrowth (PHR) are only observed when many chromosomes are added to wheat from the perennial donor species (Hayes *et al.* 2012 and unpublished). The germplasm examined was derived from wide crosses between wheat and perennial species such as *Th. intermedium, Th. elongatum,* and *Th. ponticum*. Figure 1 is a plot of many of the hybrid derivatives, partial amphiploids and amphiploids that we have examined in the field, showing the relationship between chromosome counts and ability to regrow after the first grain harvest. This figure is based on a similar figure in Hayes *et al.* (2012), but with some new lines and field data added. Some lines were able to perenniate in one or both field environments where they were grown and a few were able to regrow through four seasons (Table 1). The wheat-derived lines that were capable of perennial regrowth contained seven or more pairs of chromosomes from the perennial parent. In the case of the wheat x *Th. elongatum* derivatives, the exception seems to be CPI147232, which has 42 chromosomes but is likely to have substituted the E genome for one of the wheat genomes, just as in the durum wheat x *Th. elongatum* amphiploid (Figure 1A). The situation is even clearer for wheat x *Th. intermedium*, wheat x *Th. ponticum* and wheat x unknown *Agropyron sp.* derivatives, where only 56 chromosomes lines show significant perenniality (Figure 1B). Individual *Triticeae* genomes have seven pairs of chromosomes, and therefore, the chromosome constitutions of stable allopolyploids, both naturally occurring and synthetic amphiploids, stabilise at multiples of 14. Many fertile and stable wheat derived amphiploids have been formed at the octoploid level (2n = 56; Mujeeb-Kazi and Hettel, 1995; Jauhar, 1995; Sepsi *et al.* 2008), and it seems this is a benchmark of stability as well as a general requirement for retaining perenniality. Figure 1 also illustrates that although the wheat hybrid derivatives do not regrow with the same consistency as perennial benchmarks such as *Th. intermedium* or *S. montanum*, some do well.

TABLE 1. SUCCESSIVE GRAIN YIELDS OF HYBRID WHEAT DERIVATIVES FROM WHEAT X *TH. ELONGATUM* OR WHEAT X *TH. INTERMEDIUM*, AND THE PERENNIAL GRASS *TH. PONTICUM*, GROWN IN AUSTRALIA

The weights are means of three replicate 1 m rows. Decline in yield, of the hybrids, over time was due to plant mortality within populations.

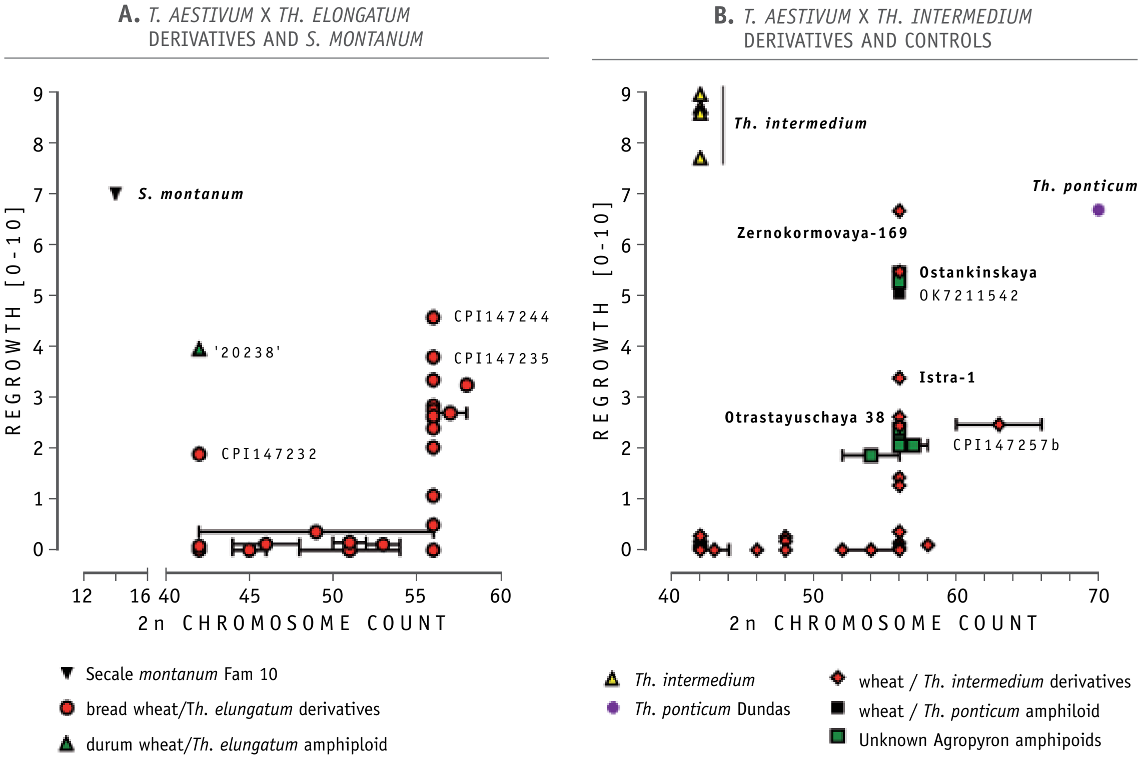
	GRAIN wt (g) 2008	GRAIN wt (g) 2009	GRAIN wt (g) 2010	GRAIN wt (g) 2011
CPI 147235a	73.5	25.9	0.0	0.0
CPI 147236a	81.7	45.9	11.2	0.0
CPI 147236b	65.9	8.9	0.0	0.0
CPI 147251b	70.9	9.4	0.5	0.4
CPI 147257b	7.8	12.6	4.7	0.7
CPI 147258a	4.7	0.3	0.3	0.0
CPI 147286a	36.3	0.4	0.0	0.0
Th. ponticum	0.0	9.4	207.7	268.8
lsd	**45.7**			

FIGURE 1. CHROMOSOME COUNTS (2) AND POST-HARVEST REGROWTH (PHR) SCORE IN THE FIELD FOR A RANGE OF CONTROL PERENNIAL SPECIES AND WHEAT HYBRID DERIVATIVES

The bars show the range where variable chromosome counts were observed. Some of the data plotted are from Hayes *et al.* (2012).

A. shows mainly wheat x *Th. elongatum* derivatives.

B. shows mainly wheat x *Th. intermedium* derivatives.



A. *T. AESTIVUM* X *TH. ELONGATUM* DERIVATIVES AND *S. MONTANUM*

B. *T. AESTIVUM* X *TH. INTERMEDIUM* DERIVATIVES AND CONTROLS

Perennial amphiploids or partial amphipoids have been produced between wheat and *Th. ponticum* (2n = 70, decaploid) (Berezhnoi, 1987; Zhang *et al.* 1996; Chen *et al.* 1998), *Leymus arenarius* (2n = 56, octoploid) (Anamthawat-Jonsson, 1995), *Th. intermedium* (2n = 42, hexaploid) (Tsitsin and Lubimova, 1959; Cauderon, 1966; Sun, 1981; Jones *et al.* 1999; Cox *et al.* 2002) and *Th. elongatum* (2n = 14, diploid) (Jenkins and Mochizuki, 1957; Rommel and Jenkins, 1959; Cai *et al.* 2001; Murphy *et al.* 2007; Mujeeb-Kazi *et al.* 2008). Taken together, this experience suggests that the best near-term prospect for a perennial wheat-like grain crop is a full or partial amphiploid, containing the full set of tetraploid (AABB) or hexaploid (AABBDD) wheat chromosomes plus one genome equivalent (XX) from the donor. If the perennial donor is a polyploidy, the extra genome in the allopolyploid (partial amphiploid) is usually a synthetic genome. In this context, "synthetic genome" means one consisting of a mixture of chromosomes from the multiple perennial parent genomes, but where each of the seven homologous chromosome groups are represented in the synthetic genome. If each of the homologous groups are not represented the synthetic genome is unstable. So, while a partial amphiploid with a synthetic genome may be stable, a significant difficulty results for any ongoing breeding program. Each time a primary partial amphiploid is produced, the synthetic genome may consist of a different mix of donor chromosomes (Jones *et al.* 1999; Han *et al.* 2004; Liu *et al.* 2005), and therefore interbreeding of primary partial amphiploids often will result in a loss of the donor chromosomes, genetic instability and consequent likely loss of the perennial trait. Banks *et al.* (1993) amply demonstrated this problem through experiments in which a set of independent primary wheat-*Th. intermedium* partial amphiploids (2n = 56) were inter-crossed. We would argue that the ability to inter-breed from independent primary amphiploids is essential to enable a practical rate of breeding progress with hybrid perennial wheats. Robust perennials might be achievable with partial amphiploids using a polyploidy perennial donor; some of the best performing lines in our experiments have derived from polyploid donors such as wheat x *Th. ponticum* (10x) derivatives or wheat x *Th. intermedium* (6x) (Figure1B). However, substantial progress will be made best, if ongoing breeding programs can be established through developing genetically diverse primary amphiploids, inter-crossing them to generate large scale genetic segregation on which to impose heavy selection.

Consequently, although perennial allopolyploid *Triticeae* might make good donors for primary partial amphiploids, we believe the best prospects for a productive breeding program in the medium term should focus on a diploid perennial donor such as *Th. elongatum* (Host) D. R. Dewey (EE, 2n = 2x = 14, synonyms *Lophopyrum elongatum, Elytrigia elongata, Elymus elongatus, Agropyron elongatum*). The Washington State University (WSU) program used this donor species introduced as the Chinese Spring wheat x *Th. elongatum* amphiploid they called AgCs (Jenkins, 1957; Cai *et al.* 2001). It is helpful to consider the analogy with the breeding of the human-made cereal triticale. If a tetraploid wheat is used, the amphiploid would be AABBEE (analogous to hexaploid triticale, AABBRR); if a hexaploid wheat is used, the amphiploid would be AABBDDEE (analogous to octoploid triticale, AABBDDRR). Lelley (2006) gives a useful summary of the history and breeding of triticale. The first fertile triticale, an octoploid, was produced by Rimpau in 1888. It was only in the 1930s, that induced chromosome doubling techniques using colchicine enabled routine production of fertile primary triticales. Research switched to hexaploid triticales in the 1950s, and progress was made by extracting secondary hexaploids from crosses between hexaploid and octoploid triticales. The International Maize and Wheat Improvement Center (CIMMYT) achieved another step forward by producing hexaploid cv. Armadillo carrying a 2D (2R) chromosomal substitution, though subsequent developments have shown that the full potential of triticale is best served with a full rye genome and without substitutions. Decades of breeding and selection have been required to produce high yielding triticale cultivars. There is evidence that during this time genomic sequence modifications were important (Ma and Gustafson, 2008; Tang *et al.* 2008; Ozkan and Feldman, 2009; Feldman and Levy, 2012). The observed changes were mainly losses of Amplified fragment length polymorphism (AFLP) and Restriction fragment length polymorphism (RFLP) bands, predominantly from the rye genome and largely involving repetitive DNA sequences. A positive relationship was found between increased chromosome bivalent pairing in meiosis, improved fertility and elimination of low-copy non-coding DNA sequences (Ozkan and Feldman, 2009). These chromosomal rearrangements may have facilitated the wheat and rye genomes coordinating the timing of their division processes during meiotic division. Both genetic and epigenetic changes have been demonstrated to occur in newly synthesized allotetraploid wheat lines with diverse genome compositions (Qi *et al.* 2010). Some of the changes seemed to be required, which they called *directed*, while others were highly variable, which they called *stochastic*. Feldman and Levy (2012) prefer the word *revolutionary* to describe rapid changes required in new allopolyploids. The changes observed included non-random loss of non-coding, low- and high-copy sequences, resulting in a DNA loss of 2-10 percent compared with the sum of the parental DNA contents. In the case of triticale, the loss was about 9 percent in octoploid triticale and 28-30 percent in hexaploid triticale, compared with the wheat and rye parental DNA. Intergenomic invasion by sequences such as transposons, and reduction in repeats of Ribosomal ribonucleic acid (rRNA) genes are also noted as revolutionary genetic changes occurring rapidly after allopolyploidisation. Epigenetic (DNA methylation) changes were also observed. More recently Hu *et al.* (2012) looked specifically at newly formed wheat x *Th. elongatum* amphiploids using genome specific molecular markers and found chromosome rearrangements and sequence duplications occurring. Interestingly, the whole genome shot-gun sequencing of wheat has revealed the apparent dynamism of hexaploid wheat genomes, in which there has been significant loss of members of multigene families during polyploidization and domestication (Brenchley *et al.* 2012).

Most of the wheat x *Th. elongatum* amphiploids we have examined have been from WSU and are octoploid ($2n = 56$, presumably AABBDDEE). However, one of the perennial WSU lines, CPI147232, is hexaploid ($2n = 42$). Its genomic composition needs to be confirmed.

Another hexaploid amphiploid is derived from cv. Stewart (*T. turgidum*) x *Th. elongatum* line (AABBEE) (Jenkins and Mochizuki, 1957). This hexaploid is too tall but should be crossable to CPI147232, provided the latter is also AABBEE, in which case the progeny should segregate to form populations at the hexaploid level useful for selections. Almouslem and Amleh (1999) also report a durum wheat x *Th. elongatum* hybrid. The greater genomic proportion of the perennial genome to annual genomes may confer a more dominant perennial habit; however this remains to be tested.

More recently we have obtained new wheat x *Th. elongatum* amphiploids at the octoploid level from CIMMYT, in particular two lines using wheat cv. Goshawk (Mujeeb-Kazi *et al.* 2008). These had good semi-dwarf stature, strong straw and reasonable PHR in the greenhouse. However, fertility was reduced and the heads were very susceptible to shattering (Larkin, unpublished). Shattering was not a problem with the perennial wheat *Th. elongatum* amphiploids from WSU. Crosses between the two types have been successful. And hopefully will allow the recovery of progeny with non-shattering heads, but with semi-dwarf stature and improved fertility. *Th. elongatum* accessions are available from dry and hot locations such as Israel, North Africa and Mediterranean France. Although this diploid species is not endemic in Australia, some accessions may exhibit the type of summer survival and persistent perenniality required. These should be exploited to make new primary amphiploids with adapted annual wheats.

Th. elongatum is not the only perennial diploid that might serve as the donor of the perennial habit to wheat. It is noteworthy that most species of the *Triticeae* are perennial (Barkworth *et al.* 2009) with about ten basic genome types represented, including *Australopyrum retrofractum* (W), *Agropyron cristatum* (P), *Thinopyrum bessarabicum* (Eb), *Dasypyrum villosum* (V), *Psathyrostachys hushanica* (Ns), *Pseudoroegneria spicata* (St); and wheat can be hybridised to some of these species. So there should be many other possibilities for generating perennial amphiploids. The diploid *Australopyrum* species (2n = 2x = 14, WW) might appear to be an attractive donor of perenniality in an Australian context. However, there are no reports of hybrids with wheat. Furthermore, *Australopyrum* species lack the broader adaptation to various climatic regions of southern Australia associated with the other native perennial *Triticeae* species, *Elymus scaber*.

Some attention has been given to producing a perennial triticale derived from perennial rye, *Secale montanum,* instead of cereal rye to combine with wheat. Schlegel (1980) produced F1 hybrids and amphiploids from wheat x *S. montanum* crosses; the amphiploids at meiosis had an average of 26.55 bivalents compared with 27.30 in an established octoploid triticale, suggesting a reasonable genetic stability early in the breeding cycle. There was a correlation between the amount of telomeric rye heterochromatin and the frequency of univalents in meiosis, suggesting the heterochromatin difference between the wheat and rye chromosomes was an impediment to full fertility. Delayed DNA replication at the heterochromatic telomeres may be a problem for coordinating the timing of wheat and rye chromosome division processes during meiotic division. The reduced amount of telomeric heterochromatin in *S. montanum* relative to *S. cereale* is postulated to explain why wheat *S. montanum* amphiploids were more regular in pairing than primary wheat *S. cereale* amphiploids (Thomas and Kaltsikes, 1974; Schlegel, 1980). Established fully fertile triticales have lost much of the telomeric heterochromatin on the rye chromosomes. This is another example of a genetic change required to enable newly formed allopolyploids to be fully fertile.

There has been some success in breeding perennial rye itself as a grain crop through intercrossing *S.cereale* and *S. montanum* and the release of tetraploid cvs. Permontra and Sopertra, diploid cv. Benmonta in Germany (Reimann-Philipp, 1995), and diploid cvs. Perenne and Kriszta in Hungary (Kotvics *et al.* 2001; Füle *et al.* 2005; Sipos and Halasz, 2007). In Canada, cv ACE-1 was developed by selection from German germplasm (Acharya *et al.* 2004). Initial attempts had difficulties with fertility and retention of perenniality. Success came only after decades of selection. In Australia, a perennial rye cv. Black Mountain was bred from a *S. cereale x S. montanum* cross with two backcrosses to the perennial parent; selection for this release was based mainly on perenniality and forage yield, rather than grain yield. Following cell culture and six generations of selection for fertility, non-shattering heads and grain yield, a higher grain yield perennial selection was recovered called Family 10 (Oram, 1996; personal communication, 2010).

CONCLUSIONS

With the increasing power and reach of genomic analyses, greater genetic insight will come eventually into the genes controlling the perennial habit. Transcriptomic comparisons between annual and perennial *Brachypodium* species, being arguably the closest model species to wheat, might prove particularly informative. Other potentially informative comparisons include annual and perennial rice (*Oryza rufipogon*) (Zhao *et al.* 2012), *Sorghum bicolor* and *S. halepense*, *Hordeum vulgare* and *H. bulbosum*, *Panicum miliaceum* and *P. turgidum*. Eventually such studies could lead to the ability to engineer perenniality into wheat through transgenics or genome editing. In the meantime we would contend that the best near term prospect of a productive breeding program for a perennial wheat-derived cereal will involve the following steps:

1. The generation of many primary amphiploids between wheat and a perennial diploid such as *Th. elongatum*. Importantly, this should involve a diversity of *Th. elongatum* accessions and a diversity of annual wheat cultivars adapted to various target zones globally.
2. Intercrossing primary amphiploids and advancing segregating populations with selection.
3. Early generation selection at F2 to F4 would emphasise traits such as semi-dwarf plant height, non-shattering heads, stable amphiploid chromosome count, large seed size, good self-fertility, and regrowth in pots.
4. Later generation selections would emphasise traits such as maturity, robust post-harvest growth, grain yield, forage yield, stability of grain yield across seasons, and disease resistance.

In addition to a number of rounds of such breeding and trait selection cycles, progress might be further accelerated through specific attention and selection for the *revolutionary* genetic changes so frequently observed in newly forming allopolyploids (Feldman and Levy, 2012), that signal the accommodation between genomes and the rise in fertility and stability. While triticale took over 40 years to begin to deliver on its potential, the time required to deliver a successful perennial wheat might be greatly compressed through a coordinated international effort and application of our greater understanding of the genomic changes required before a newly formed allopolyploid becomes stable and productive. International cooperation could be encouraged through the formation of a *community-of-practice* with mutually agreed *open-source* style terms of engagement. We already have the beginnings of such a community. High priority should be given to collecting diverse accessions of diploid *Th. elongatum*, crossing to wheat and forming amphiploids, sharing diverse primary amphiploids, intercrossing to form secondary amphiploids, and coordinated multi-environment testing of derived secondary amphiploid populations.

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REFERENCES

Acharya, S.N., Mir, Z. & Moyer, J.R. 2004. ACE-1 perennial cereal rye. *Canadian Journal of Plant Science* 84: 819-821.

Almouslem, A.B. & Amleh, N. 1999. An intergeneric hybrid between durum wheat and diploid wheatgrass *Lophopyrum elongatum* (Host) A-Love. *Kuwait Journal of Science & Engineering* 26: 143-155.

Anamthawat-Jonsson, K. 1995. Wide-hybrids between wheat and lymegrass: breeding and agricultural potential. *Buvisindi Icel. Agr. Sci*. 9: 101-113.

Banks, P.M., Xu, S.J., Wang, R.R.C. & Larkin, P.J. 1993. Varying chromosome composition of 56-chromosome wheat x *Thinopyrum intermedium* partial amphiploids. *Genome*. 36: 207-215.

Barkworth, M.E., Cutler, D.R., Rollo, J.S., Jacobs, S.W.L. & Rashid, A. 2009. Morphological identification of genomic genera in the *Triticeae*. *Breeding Science.* 59: 561-570.

Bell, L.W., Byrne, F., Ewing, M.A. & Wade, L.J. 2008. A preliminary whole-farm economic analysis of perennial wheat in an Australian dryland farming system. *Agricultural Systems.* 96: 166-174.

Bell, L.W., Ryan, M.H., Bennett, R.G., Collins, M.T. & Clarke, H.J. 2012. Growth, yield and seed composition of native Australian legumes with potential as grain crops. *Journal of the Science of Food and Agriculture.* 92: 1354-1361.

Bell, L.W., Wade, L.J. & Ewing, M.A. 2010. Perennial wheat: a review of environmental and agronomic prospects for development in Australia. *Crop & Pasture Science.* 61: 679-690.

Berezhnoi, P. 1987. Development of the ideas of N. I. Vavilov on distant hybridization in wheat breeding. *Selektsiya i Semenovodstvo, Moscow.* 6: 49-52.

Brenchley, R., Spannagl, M., Pfeifer, M., Barker, G.L.A., D'Amore, R., Allen, A.M., McKenzie, N., Kramer, M., Kerhornou, A., Bolser, D., Kay, S., Waite, D., Trick, M., Bancroft, I., Gu, Y., Huo, N., Luo, M-C., Sehgal, S., Gill, B., Kianian, S., Anderson, O., Kersey, P., Dvorak, J., McCombie, W.R., Hall, A., Mayer, K.F.X., Edwards, K.J., Bevan, M.W. & Hall, N. 2012. Analysis of the breadwheat genome using whole-genome shotgun sequencing. *Nature.* 491: 705-710.

Cai, X., Jones, S.S. & Murray, T.D. 2001. Molecular cytogenetic characterization of *Thinopyrum* genomes conferring perennial growth habit in wheat-*Thinopyrum* amphiploids. *Plant Breeding.* 120: 21-26.

Cauderon, Y. 1966. Cytogenic study of material resulting from a cross between *Triticum aestivum* and *Agropyron intermedium*. I. Creation of stable addition lines. *Annales de l'Amelioration des plantes.* 16: 43-70.

Chen, Q., Ahmad, F., Collin, J., Comeau, A., Fedak, G., St-Pierre, C. & Chen, Q. 1998. Genomic constitution of a partial amphiploid OK7211542 used as a source of immunity to barley yellow dwarf virus for bread wheat. *Plant Breeding.* 117: 1-6.

Cox, T.S., Bender, M., Picone, C., Van Tassel, D.L., Holland, J.B., Brummer, E.C., Zoeller, B.E., Paterson, A.H. & Jackson, W. 2002. Breeding perennial grain crops. *Critical Reviews in Plant Sciences.* 21: 59-91.

Cox, T.S., Van Tassel, D.L., Cox, C.M. & Dehaan, L.R. 2010. Progress in breeding perennial grains. *Crop & Pasture Science.* 61: 513-521.

Culman, S.W., Snapp, S.S., Ollenburger, M., Basso, B. & DeHaan, L.R. 2013. Soil and Water Quality Rapidly Responds to the Perennial Grain Kernza Wheatgrass. *Agronomy Journal.* 105: 735-744.

Curtin, S.J., Voytas, D.F. & Stupar, R.M. 2012. Genome engineering of crops with designer nucleases. *Plant Genome.* 5: 42-50.

Davies, C.L., Waugh, D.L. & Lefroy, E.C. 2005. Variation in seed yield and its components in the Australian native grass *Microlaena stipoides* as a guide to its potential as a perennial grain crop. *Australian Journal of Agricultural Research.* 56: 309-316.

DeHaan, L.R., Van Tassel, D.L. & Cox, T.S. 2005. Perennial grain crops: A synthesis of ecology and plant breeding. *Renewable Agriculture and Food Systems*. 20: 5-14.

Faris, J.D., Fellers, J.P., Brooks, S.A. & Gill, B.S. 2003. A bacterial artificial chromosome contig spanning the major domestication locus Q in wheat and identification of a candidate gene. *Genetics*. 164: 311-321.

Feldman, M. & Levy, A.A. 2012. Genome Evolution Due to Allopolyploidization in Wheat. *Genetics.* 192: 763-774.

Gegas, V.C., Nazari, A., Griffiths, S., Simmonds, J., Fish, L., Orford, S., Sayers, L., Doonan, J.H. & Snape, J.W. 2010. A genetic framework for grain size and shape variation in wheat. *Plant Cell.* 22: 1046-1056.

Han, F.P., Liu, B., Fedak, G. & Liu, Z.H. 2004. Genomic constitution and variation in five partial amphiploids of wheat - *Thinopyrum intermedium* as revealed by GISH, multicolor GISH and seed storage protein analysis. *Theoretical and Applied Genetics.* 109: 1070-1076.

Hayes, R.C., Newell, M.T., DeHaan, L.R., Murphy, K.M., Crane, S., Norton, M.R., Wade, LJ., Newberry, M., Fahim, M., Jones, S.S., Cox, T.S. & Larkin, P.J. 2012. Perennial cereal crops: an initial evaluation of wheat derivatives. *Field Crops Research*. 133: 68-89.

Hu, L-J., Liu, C., Zeng, Z-X., Li, G-R., Song, X-J. & Yang. Z-J. 2012. Genomic rearrangement between wheat and *Thinopyrum elongatum* revealed by mapped functional molecular markers. *Genes & Genomics* 34: 67-75.

Jaikumar, N.S., Snapp, S.S., Murphy, K. & Jones, S.S. 2012. Agronomic assessment of perennial wheat and perennial rye as cereal crops. *Agronomy Journal.* 104: 1716-1726.

Jauhar, P. 1995. Meiosis and fertility of F1 hybrids between hexaploid bread wheat and decaploid tall wheatgrass (*Thinopyrum ponticum*). *Theoretical and Applied Genetics.* 90: 865-871.

Jenkins, B. & Mochizuki, A. 1957. A new amphiploid from a cross between *Triticum durum* and *Agropyron elongatum* (2n = 14). *Wheat Information Service.* 5: 15.

Jenkins, B.C. 1957. The addition of an Agropyron genome to the common wheat variety Chinese Spring. *Wheat Information Service.* p 14.

Jones, T.A. Zhang, X.Y. & Wang, R.R.C. 1999. Genome characterization of MT-2 perennial and OK-906 annual wheat x intermediate wheatgrass hybrids. *Crop Science.* 39: 1041-1043.

Kasema, S., Waters, D.L.E., Rice, N., Shapter, F.M. & Henry, R.J. 2010. Whole grain morphology of Australian rice species. *Plant Genetic Resources.* 8: 74-81.

Kotvics, G., Krisztian, J. & Heszky, L. 2001. Perennial Rye: A new forage crop for the world, registered in Hungary. *Hungarian Agricultural Research*. 10: 4-5.

Lammer, D., Cai, X., Arterburn, M., Chatelain, J., Murray, T. & Jones, S. 2004. A single chromosome addition from *Thinopyrum elongatum* confers a polycarpic, perennial habit to annual wheat. *Journal of Experimental Botany*. 55: 1715-1720.

Larkin, P.J., Newell, M.T., Hayes, R.C., Aktar, J., Norton, M.R., Moroni, S.J. & Wade, L.J. 2014. Progress in developing perennial wheats for grain and grazing. *Crop Pasture Science*. http://dx.doi.org/10.1071/CP13330.

Lelley, T. 2006. Triticale: A low-input cereal with untapped potential. *Genetic Resources, Chromosome Engineering, and Crop Improvement: Cereals.* 2: 395-430.

Liu, S.B., Wang, H.G., Zhang, X.Y., Li, X.F., Li, D.Y., Duan, X.Y. & Zhou, Y.L. 2005. Molecular cytogenetic identification of a wheat-*Thinopyrun Intermedium* (Host) Barkworth & Dr Dewey partial amphiploid resistant to powdery mildew. *Journal of Integrative Plant Biology.* 47: 726-733.

Ma, X-F. & Gustafson, J.P. 2008. Allopolyploidization-accommodated genomic sequence changes in triticale. *Annals of Botany.* 101: 825-832.

Malory, S., Shapter, F.M., Elphinstone, M.S., Chivers, I.H. & Henry, R.J. 2011. Characterizing homologues of crop domestication genes in poorly described wild relatives by high-throughput sequencing of whole genomes. *Plant Biotechnology Journal.* 9: 1131-1140.

Mujeeb-Kazi, A., Cortes, A., Gul, A., Farooq, M., Majeed, F., Ahmad, I., Bux, H., William, M., Rosas, V. & Delgad, R. 2008. Production and cytogenetics of a new *Thinopyrum elongatum/Triticum aestivum* hybrid, its amphiploid and backcross derivatives. *Pakistan Journal of Botany*. 40: 565-579.

Mujeeb-Kazi, A. & Hettel, G.P. 1995. Utilizing wild grass biodiversity in wheat improvement: 15 years of wide cross research at CIMMYT. *CIMMYT Research Report*. xxiv + 140 pp.

Murphy, K.M., Carter, A., Zernetra, R.S. & Jones, S.S. 2007. Karyotype and ideogram analyses of four wheatgrass cultivars for use in perennial wheat breeding. *Journal of Sustainable Agriculture.* 31: 137-149.

Murphy, K.M., Hoagland, L.A., Reeves, P.G., Baik, B.K. & Jones, S.S. 2009. Nutritional and quality characteristics expressed in 31 perennial wheat breeding lines. *Renewable Agriculture and Food Systems*. 24: 285-292.

Murphy, K.M., Lyon, S.R., Balow, K.A. & Jones, S.S. 2010. Post-sexual cycle regrowth and grain yield in *Thinopyrum elongatum x Triticum aestivum* amphiploids. *Plant Breeding*. 129: 480-483.

O'Neill, G. 2007. Forgotten treasures. *Ecos.* 135: 8-11.

Oram, R.N. 1996. *Secale montanum* - A wider role in Australasia? *New Zealand Journal of Agricultural Research*. 39: 629-633.

Ozkan, H. & Feldman, M. 2009. Rapid cytological diploidization in newly formed allopolyploids of the wheat (*Aegilops-Triticum*) group. *Genome*. 52: 926-934.

Pearlstein, S.L., Felger, R.S., Glenn, E.P., Harrington, J., Al-Ghanem, K.A. & Nelson, S.G. 2012. Nipa (*Distichlis palmeri*): A perennial grain crop for saltwater irrigation. *Journal of Arid Environments.* 82: 60-70.

Peleg, Z., Fahima, T., Korol, A.B., Abbo, S. & Saranga, Y. 2011. Genetic analysis of wheat domestication and evolution under domestication. *Journal of Experimental Botany.* 62: 5051-5061.

Peng, J.H.H., Sun, D.F. & Nevo, E. 2011. Domestication evolution, genetics and genomics in wheat. *Molecular Breeding* 28: 281-301.

Qi, B., Zhong, X., Zhu, B., Zhao, N., Xu, L., Zhang, H., Yu, X & Liu, B. 2010. Generality and characteristics of genetic and epigenetic changes in newly synthesized allotetraploid wheat lines. *Journal of Genetics and Genomics*. 37: 737-748.

Reeling, C.J., Weir, A.E., Swinton, S.M. & Hayes, R.C. 2012. A comparitive breakeven net return threshold to guide development of conservation technologies with application to perennial wheat. *Agriculture and Applied Economics Association's Annual Meeting*.

Reimann-Philipp, R. 1995. Breeding perennial rye. *Plant Breeding Reviews* 13: 265-292.

Rommel, R. & Jenkins, B.C. 1959. Amphiploids in *Triticinae* produced at the University of Manitoba from March 1958 to December 1959. *Wheat Information Service.* p-23.

Sang, T. 2009. Genes and mutations underlying domestication transitions in grasses. *Plant Physiol.* 149: 63-70.

Schlegel, R. 1980. Amphidiploid hybrids from crosses of hexaploid wheat with several species of rye and the relationship between the amount of heterochromatin and meiotic chromosome pairing. *Hodowla Roslin, Aklimatyzacja i Nasiennictwo*. 24: 307-314.

Sepsi, A., Molnar, I., Szalay, D., Molnar-Lang, M. 2008. Characterization of a leaf rust-resistant wheat-*Thinopyrum ponticum* partial amphiploid BE-1, using sequential multicolor GISH and FISH. *Theoretical and Applied Genetics.* 116: 825-834.

Sipos, T., & Halasz, E. 2007. The role of perennial rye (*Secale cereale* x *S. montanum*) in sustainable agriculture. *Cereal Research Communications*. 35: 1073-1075.

Sun, S. 1981. The approach and methods of breeding new varieties and new species from Agrotriticum hybrids. *Acta Agronomica Sinica*. 7: 51-57.

Suneson, C., El Sharkawy, A., & Hall, W.E. 1964. Progress in 25 years of perennial wheat breeding. *Crop Science*. 3: 437-439.

Takahashi, Y., & Shimamoto, K. 2011. Heading date 1 (Hd1), an ortholog of Arabidopsis CONSTANS, is a possible target of human selection during domestication to diversify flowering times of cultivated rice. *Genes & Genetic Systems*. 86: 175-182.

Tang, Z.X., Fu, S.L., Ren, Z.L., Zhou, J.P., Yan, B.J., & Zhang, H.Q. 2008. Variations of tandem repeat, regulatory element, and promoter regions revealed by wheat-rye amphiploids. *Genome*. 51: 399-408.

Thomas, J. & Kaltsikes, P. 1974. Possible effect of heterochromatin on chromosome pairing. *Proceedings of the National Academy of Sciences of the United States of America*. 71: 2787-2790.

Tsitsin, N.V. & Lubimova, V.F. 1959. New species and forms of cereals derived from hybridization between wheat and couch grass. *American Naturalist*. 93: 181-191.

Van Tassel, D.L., DeHaan, L.R. & Cox, T.S. 2010. Missing domesticated plant forms: can artificial selection fill the gap? *Evolutionary Applications*. 3: 434-452.

Zhang, X., Dong, Y. & Wang, R.R-C. 1996. Characterization of genomes and chromosomes in partial amphiploids of the hybrid *Triticum aestivum* × *Thinopyrum ponticum* by *in situ* hybridization, isozyme analysis, and RAPD. *Genome*. 39: 1062-1071.

Zhao, X., Zhang, T., Huang, L., Wu, H., Hu, F., Zhang, F., Zhu, L. & Fu, B. 2012. Comparative metabolite profiling and hormone analysis of perennial and annual rice. *Journal of Plant Biology*. 55: 73-80.

05

EVALUATION OF NINE PERENNIAL WHEAT DERIVATIVES GROWN IN ITALY

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ABSTRACT

As part of an international network coordinated by the Australian NSW Department of Primary Industries, nine lines of perennial wheat obtained from crosses between *Triticum aestivum* and *Thinopyrum* spp. were grown at Montelibretti (Rome) in randomized blocks with three replications during two years of testing, and compared for their agronomical, nutritional and technological properties with common wheat cultivars (cvs) Wedgetail and Enesco. All perennial genotypes were characterized by post-harvest regrowth (PHR), lateness of ear emergence, small kernels, loose spikes, variable number of seeds/spikes and high number of tillers. In addition, perennial lines had medium test weight, low percentages of hull-less kernels, high protein content, reduced sodium dodecyl sulphate (SDS) sedimentation volume and kernel texture typical of soft or medium-hard wheat. The hard-textured lines showed novel genes coding for puroindolines A and B inherited from wheatgrass (*Thinopyrum* spp.). Analysis of single seeds revealed a marked inter- and intra-line variation for gliadins and HMW-glutenin subunits (HMW-GS). The total content in bioactive compounds 5-n-alkylresorcinols and soluble polyphenols (SP) was high in perennial lines compared with their annual counterparts. Furthermore, perennial lines exhibited high yellow pigment content and resistant-starch percentage. The poor gluten quality of some perennial lines was associated with the presence of prolamins inherited from the wheatgrass parent and the absence of high-quality, HMW-GS from the wheat parent. Evidence was obtained that chromosome substitution or allosyndetic recombination between E-genome and ABD-genome chromosomes likely occurred in some perennial lines with *Th. elongatum* in their pedigree. The perennial genotypes were found to be valuable for their PHR potential and nutritional value. However, they deserve closer attention for some negative agronomical and quality traits.

Keywords: bioactive compounds, gluten quality, perennial wheat, puroindolines, storage proteins

INTRODUCTION

In the last few decades, one third of Earth's arable land has been lost due to erosion (Pimentel *et al.* 1995) and the production systems based on annual grain crops such as wheat, maize, rice and soybean have been considered among the primary causes of this soil erosion (Glover, 2005). By contrast, the high productive potential and the efficient use of natural resources such as light, water, CO_2, nitrogen compounds and minerals by perennial plant communities (Crews, 2005) suggest that perennial grain crops could meet the increasing demands of food while reducing soil erosion. Moreover, perennial grain crops are seen as an opportunity to improve water, minerals and fertilizers management, while increasing biodiversity, underground biomass and carbon sequestration in the soil (DeHaan *et al.* 2005; Jordan *et al.* 2007). Perenniality seems to be under the control of multiple genes responsible for specific biological structures such as bulbs, rhizomes and meristems, as well as for physiological traits such as resistance to cold, drought and biotic stresses. Therefore, transformation of annual crops into perennial crops with high grain yield, coupled with superior technological and nutritional quality could turn out to be a very demanding and challenging goal. In addition, annual species supply much of the photosynthetic energy for seed development, whereas perennial species allocate a proportion of the photosynthate to their roots and green tissues late in the growing season, after the annuals have senesced. This "energy tradeoff" between grain and perennating structures would result in decreased grain production per hectare of perennial species as compared with their annual counterparts (Wagoner, 1990). However, the biological superiority of annual species in producing a high amount of seed could be the result of their evolutionary history and selection, both natural and human-oriented, rather than an integrant consequence of their annual habit (DeWet, 1981; DeHaan *et al.* 2005). On the other hand, in certain conditions, the decreased input costs of a perennial grain crop can make up the difference in profit and provide additional ecosystem services (Bell *et al.* 2008).

Common wheat (*Triticum aestivum*) is the most widespread annual grain crop grown on more than 220 million hectares. Cultivation of this cereal species is claimed to be one of the primary causes of soil erosion, with annual losses of soil as high as 31.5 tonnes/ha in the case of wheat monocultures (Reganold *et al*. 1987).

The earliest forms of perennial wheat were produced in Russia in the second decade of the last century (Tsitsin and Lubinova, 1959), whereas more recent material has been developed by The Land Institute and Washington State University in the United States (Cox *et al*. 2010; Murphy *et al*. 2010). These perennial genotypes derive from crosses between wheat and one of three species of *Thinopyrum*, namely (i) *Th. elongatum* (synonymous *Agropyrum elongatum*), a diploid species ($2n = 2x = 14$) with the E genome, (ii) *Th. intermedium* (synonyms *Ag. intermedium*, *Elymus hispidus* or *Elytrigia intermedia*), an hexaploid species ($2n = 6x = 42$) with the $JJ^{S}S$ genome, and (iii) *Th. ponticum* (synonyms *Ag. ponticum*, *Elymus elongatus* spp. *ponticus* or *Elytrigia pontica*), a decaploid species ($2n = 10x = 70$) with three copies of the J genome and two copies of the J^{s} genome. In some cases, tetraploid AB-genome wheat *T. turgidum* ssp *carthlicum* was used as a bridge species with *Th. intermedium*, and the hybrid progeny was crossed with common wheat.

Among 176 wheat genotypes with wheatgrass (*Thinopyrum* spp.) in the pedigree, 107 lines demonstrated some ability to regrow after the sexual cycle PHR over three consecutive years of cultivation in two Australian locations (Hayes *et al.* 2012). As part of an international network of field trials coordinated by the Australian NSW Department of Primary Industries, nine of the wheat x wheatgrass derivatives with a relatively high PHR capacity mentioned above were seeded in Italy in 2011. The present study aimed to compare these lines with two commercial common wheat cultivars for their agronomical, technological and nutritional traits during two years of testing.

MATERIALS AND METHODS

Plant material

Nine perennial wheat breeding lines kindly provided by Richard Hayes (Graham Centre for Agricultural Innovation, NSW, Wagga Wagga, Australia) were grown at Montelibretti (Rome) in the Tiber valley on sandy soil, with an average annual rainfall of 954 mm. Plants were sown in December 2011 in 1 m rows, 0.5 m apart, with 30 kernels/row in a randomized block experimental design with three replications. Two annual common wheat cultivars, the Australian cv. Wedgetail and the Italian cv. Enesco, were used as controls. Perennial accessions of *Secale montanum*, *Hordeum bulbosum*, *Thinopyrum ponticum* and *Th. intermedium* were included in the experiment as well. The full list of entries is given in Table 1. At sowing, 31 Kg/ha nitrogen and 20 Kg/ha of phosphorus were applied in the form of urea and diammonium phosphate. The date of anthesis was recorded and maturity of each genotype was calculated as time between sowing and anthesis. At harvest, length of the main stem and total number of tillers of each plant were recorded and plants were cut at approximately 10 cm from the soil surface. In addition, spikes from the main stems were threshed in a bench micro-thresher to determine percentage of hull-less kernels, number of kernels per spike, 1 000-kernel weight and test weight. Rows were visually monitored for signs of regrowth every four weeks for three months from harvest and each genotype received a PHR score based on the average percentage of the original plant population that was regrowing in the three replicates.

TABLE 1. PEDIGREE, CHROMOSOME NUMBER AND POST-HARVEST REGROWTH (PHR) OF NINE WHEAT X WHEATGRASS DERIVATIVES

WHEAT DERIVATIVES	PEDIGREE AND ORIGIN	NO. OF CHROMOSOMES[c]
235A	*Th. elongatum / T. aestivum*[a] // *T. aestivum*[b] (WSU)	42, 44, 56
236A	*Th. elongatum / T. aestivum*[a] // *T. aestivum*[b] (WSU)	56, 58
244B	*Th. elongatum / T. aestivum*[a] // *T. aestivum*[b] (WSU)	56
251B	*Th. elongatum / T. aestivum*[a] // *T. aestivum*[b] (WSU)	56
280B	*Th. intermedium / T. carthlicum // T. aestivum* (TLI)	56
281B	*Th. intermedium / T. carthlicum // T. aestivum* (TLI)	56
11955	*Triticum* spp./ *Thinopyrum* spp.(USA)	56
OK72	*T. aestivum/Th. ponticum* (USA)	56
OT38	*T. aestivum/Th. intermedium* (ex USSR)	56

a Cv. Chinese Spring
b Cv. Madsen
c According to Hayes *et al.* (2012)
WSU = Washington State University
TLI = The Land Institute

Technological analyses

Analyses were performed on wholemeal from mature kernels of each replication ground with a laboratory mill (Cyclotec, mod. 1093-Tecator/Hoganas, Sweden) equipped with a 1.0 mm sieve. Protein content was determined by micro-Kjeldhal nitrogen analysis (N x 5.7), whereas gluten quality was evaluated by the SDS sedimentation test using a solution of 2 percent sodium dodecyl sulfate as described by the standard method 56-70 (AACC, 1995), and the sedimentation volumes were expressed in milliliters. The sequential extraction of protein in the wholemeal was carried out according to Wang *et al.* (2007). Kernel hardness was evaluated on 50 hull-less kernels by the Perten Single Kernel Characterization System (SKCS) 4100 (Springfield, IL, USA) following the manufacturer's operating procedure. The instrument was set in a range of hardness between -40 and +120.

Puroindoline and storage protein analysis

DNA was extracted from leaves by the cetyltrimethyl ammonium bromide (CTAB) method and puroindoline genes were amplified by PCR as described by Gautier *et al.* (1994). Puroindolines were extracted with 50 mM NaCl and 50 percent (v/v) propan-2-ol from 50 mg of air-dried starch granules as described previously (Corona *et al.* 2001). Fractionation of puroindolines by acidic poly-acrylamide gel electrophoresis (A-PAGE) at pH 3.1 was carried out as described by Corona *et al.* (2001). Gliadins and total proteins were extracted and fractionated by A-PAGE and SDS-PAGE, respectively, as described by Pogna *et al.* (1990).

Extraction of phenolic compounds and alkylresorcinols

Immediately after harvest, grain samples from two replicates were milled with a laboratory cyclone mill (Cyclotec 1093, Foss, Italy) to pass through a 0.5 mm sieve and kept at 4°C until extraction and analysis. All determinations were carried out in triplicate on two independent aliquots of each composite sample. Moisture content was determined at 120°C with a thermobalance (Sartorius MA 40, Gottingen, Germany).

Samples (1 g) of wholemeal were extracted to determine SP compounds and 5-n-alkylresorcinol (AR) content. Samples were placed in 50 mL tubes and extracted with 40 mL acetone for 24 hours by continuous mechanical shaking at room temperature. The extracts were then filtered through a Whatman n.42 filter paper and evaporated to dryness at 60°C in a rotary evaporator (Buchi R-114, Switzerland). The dry residues were then dissolved in pure methanol (1 mL) and immediately analysed. All reagents were of analytical spectrophotometric grade (Carlo Erba, Rome, Italy).

Determination of total soluble phenolic compounds (TSPCs)

SP of wholemeal extracts were determined using the Folin–Ciocalteu (F-C) method as reported by Moore and Yu (2008). SP content was calculated from a calibration curve, using (+) catechin as standard. Results were expressed as micrograms of catechin equivalents per gram of wholemeal sample.

Gas chromatography-mass spectrometry (GC-MS) analysis of alkylresorcinols

Total AR content in wholemeal extracts was determined by GC-MS analysis according to Landberg *et al.* (2009) using methyl behenate as internal standard. The AR extract (10 mL) of each sample was dried under nitrogen and a mixture (400 µL) of pyridine and TMCS (9:1, v/v) was added. The mixture was then shaken and heated at 70°C for 60 min. GC-MS analysis was performed with a Perkin Elmer gas chromatograph GC Clarus 600 series coupled to the mass spectrometer Clarus 580D (Perkin Elmer, Milan, Italy) equipped with a split/splitless injector, a RTX-5MS column (0.25 mm 30 m, 0.25 mm film thickness, Restek, Milan, Italy) and a quadrupole mass spectrometer (Clarus 580D, Perkin Elmer, Milan, Italy) operating in electronic impact (EI) ionisation mode (70 eV). The chromatographic conditions employed were reported by Bellato *et al.* (2013). AR content was determined by comparing the relative retention times with those obtained for a mix of the AR homologue standards C15:0, C17:0, C19:0, C21:0, C23:0 and C25:0. Chromatographic peak areas of the AR homologues in each sample were summed to yield total AR content.

Total dietary fibre, resistant starch and yellow pigments

Total dietary fibre (DF) content was determined using an enzymatic-gravimetric method (AOAC, 1995) and an automatic filtration of the hydrolysed products (Fibertec system, FossItalia, Italy). Total and resistant starch (RS) contents were evaluated by enzymatic methods using Megazyme kits, K-TSTA and K-RSTAR (Mc Cleary *et al.* 1997; Mc Cleary and Monaghan, 2002; Mc Cleary *et al.* 2002). Total yellow pigment (YP) content was determined following the AOAC 14045 (1975) method and expressed as ppm of β-carotene.

Statistical analysis

As reported by Li *et al.* (2009), two independent aliquots of composite wholemeal sample were considered as statistical replicates of each genotype. Analysis of variance was performed with the Microcomputer Program for the Design, Management, and Analysis of Agronomic Research Experiments (MSTATC) program (Michigan State University, East Lansing, MI). Simple correlation coefficients were calculated as well.

RESULTS

Post-Harvest Regrowth

Three months after the first harvest in 2012, the PHR of the nine wheat x wheatgrass derivatives varied between 5.6 and 42.1 percent, without any evident association between PHR score and genetic origin (Table 2). In some genotypes, new plants arose at the level of the ground surface or immediately below, while in others they emerged at the level of the first or the second internode. The accessions of *Thinopyrum intermedium*, *Th. ponticum* and *Hordeum bulbosum* showed PHR scores between 60.0 and 87.8 percent, approximately double that of the best perennial wheat derivatives. *Secale cereale* had a moderate (23.8 percent) PHR score, whereas annual common wheat cvs Wedgetail and Enesco did not exhibit any sign of regrowth. In 2013, the perennial wheat derivatives in the three months following the second harvest revealed a modest decline in their PHR scores with respect to the those recorded in 2012 (Table 2).

Morphological and physiological traits

The perennial wheat derivatives proved to be significantly different for all the morpho-phisiological traits analysed with respect to cvs Wedgetail and Enesco (Table 2). On average, the nine lines were characterized by lateness of ear emergence (20-30 days later compared with annual varieties), high number of tillers (13.4 *vs* 7.3, except line 236A), tall plants (88.6 *vs* 70.2 cm), loose spikes (1.13 *vs* 1.79 spikelets/cm), reduced number of kernels per spike (39.8 *vs*

63.0) and small kernels (21-33 mg *vs* 40-41 mg). However, lines 280B and 281B, which have in their pedigree the bridge tetraploid species *Triticum turgidum* ssp. *carthlicum*, were comparable with cvs Wedgetail and Enesco for plant height and ear length. In addition, the perennial wheat derivatives revealed mean test weights intermediate between those of annual controls Wedgetail (66.6 kg/hL) and Enesco 74.2(kg/hL), with the only exception of lines 244B and 280B, which showed test weights lower than 58.5 Kg/hL. Upon a single passage through a micro-thresher, spikes of perennial wheat genotypes released a low percentage of hull-less kernels (20.7 to 63.0 percent) compared with control cultivars (84.9 and 91.8 percent). Leaves and stems of perennial wheat derivatives showed no damage due to powdery mildew and rusts (*Puccinia* spp.), with the only exception being lines 251B and 236A, which revealed slight symptoms of stem rust (*Puccinia graminis tritici*). By contrast, line 244B was heavily attacked by *Helminthosporium* spp.

TABLE 2. AGRONOMIC TRAITS AND POST-HARVEST REGROWTH (PHR) OF NINE PERENNIAL WHEAT DERIVATIVES, TWO COMMON WHEAT CULTIVARS AND FOUR PERENNIAL CEREAL SPECIES[a]

GENOTYPE	HEADING TIME*	NO. OF TILLERS	PLANT HEIGHT (CM)		SPIKE LENGTH (CM)	NO. OF SPIKELETS/ SPIKE	NO. OF SPIKELETS/ (CM)	NO. OF KERNELS/ SPIKE	KERNEL WT (MG)	TEST WT (KG/ HL)	HULL-LESS KERNELS (%)	PHR (%)	
			2012	2013								2012	2013
235A	155.0c	10.6d	94.6b	139.3a	15.7a	18.5b	1.18b	59.8b	24.5d	71.2bc	46.0ef	28.1d	25.0d
236A	150.0d	7.1e	85.7c	138.5a	15.3ab	16.5c	1.08b	43.9e	24.8d	68.9c	50.3de	11.1f	9.2f
244B	157.7b	11.2d	80.5d	90.0f	14.0b	15.7c	1.13b	13.1g	23.2de	58.3e	38.8g	5.6g	5.6g
251B	150.0d	11.4d	95.8b	133.0b	16.7a	16.5c	0.98b	58.4b	25.2d	70.2c	41.7fg	36.1b	33.3c
280B	147.0e	12.1cd	64.5f	114.3e	9.8d	11.7e	1.19b	18.5f	21.4e	58.2e	25.0h	20.6e	17.6e
281B	142.3f	18.7a	79.1d	119.3d	10.3cd	13.3de	1.29b	9.2h	32.9b	70.0c	20.7h	40.5a	37.8b
11955	146.3e	15.6b	102.3a	130.5b	15.7a	15.6c	0.99b	53.5c	30.1c	68.7c	48.3de	33.3c	33.3c
OK72	141.3f	19.6a	98.4b	126.2c	15.2ab	14.8cd	0.98b	53.2c	28.3c	74.0a	51.7d	42.1a	42.1a
OT38	160.0a	14.4bc	96.8b	127.7c	15.9a	20.8a	1.31b	48.7d	23.5de	72.0b	63.0c	35.0b	30.0c
Mean	150.0	13.4	88.6	124.3	14.3	15.9	1.13	39.8	26.0	68.0	42.8	27.8	25.9
Wedgetail	129.3g	7.5e	70.3e	-	11.5c	20.7a	1.79a	63.2a	39.9a	66.6d	84.9b	0.0	0.0
Enesco	120.1h	7.0e	70.0e	-	11.1c	21.0a	1.80a	62.8a	41.0a	74.2a	91.8a	0.0	0.0
Th. intermedium	171.2	20.3	129.8	167.7	33.1	26.1	0.79	nd	nd	nd	nd	66.7	60.0
Th. ponticum	193.4	22.1	166.7	194.0	29.7	22.8	0.77	nd	nd	nd	nd	80.0	80.0
S. montanum	133.4	7.7	130.1	142.3	12.0	18.8	1.57	nd	nd	nd	nd	23.8	19.0
H. bulbosum	120.1	14.3	160.8	170.7	11.3	17.6	1.56	nd	nd	nd	nd	87.8	87.8

[a] Agronomic traits of plants harvested in 2012 (first harvest); plant height and PHR were recorded in 2013 (second harvest) as well. *Number of days from sowing.

nd, not determined. In each column, means followed by the same letter do not differ significantly from one another (Duncan test at P<0.05).

Quality traits

Protein contents as high as 19.7 to 23.7 percent were observed in the perennial wheat derivatives, with an average value of 20.6 percent, 3.3 percentage units higher than those of their annual counterparts (Table 3). On average, the perennial wheat derivatives revealed a high proportion of gliadins (37.2 percent of total proteins *vs* 33.0 percent in cv. Wedgetail) coupled with a significantly low proportion of HMW-GS (on average 9.3 percent of total protein *vs* 10.7 percent in cv. Wedgetail). This was particularly evident in lines 235A, 236A, 244B and 251B developed at the Washington State University, and was associated with poor gluten quality as determined by the SDS sedimentation test, line 235A being unique in showing an SDS sedimentation volume as high as 58 ml. The contrasting behaviour of these lines was likely due to their HMW-GS, which are known to play an important role in the visco-elastic properties of dough.

TABLE 3. PROTEIN CONTENT, PROPORTION OF FOUR PROTEIN FRACTIONS AND SDS SEDIMENTATION VOLUME OF NINE PERENNIAL WHEAT DERIVATIVES AND TWO COMMON WHEAT CULTIVARS

GENOTYPE	PROTEIN CONTENT %	ALBUMIN & GLOBULIN %	GLIADIN %	HMW-GS %	LMW-GS %	SEDIMENTATION VOLUME (ml)
235A	19.7	11.1	39.8	9.7	19.4	58c
236A	19.4	15.1	41.9	7.4	15.4	45f
244B	23.7	15.1	42.7	8.0	13.7	30i
251B	19.9	9.3	41.8	9.7	18.8	43g
280B	20.8	14.3	24.8	12.0	27.1	50d
281B	21.5	10.4	35.1	11.2	22.4	41h
11955	19.7	24.3	31.5	7.9	18.5	50d
OK72	20.6	10.9	38.1	10.1	20.0	50d
OT38	20.7	17.3	38.2	8.1	16.3	47e
Mean	20.6	14.2	37.2	9.3	19.1	46
Wedgetail	17.3	14.2	33.0	10.7	18.8	67b
Enesco	17.4	nd	nd	nd	nd	79a
F value	**	ns	***	**	ns	

**, ***Significant at $P<0.05$ and $P<0.01$ respectively; ns, not significant. In the last column, means followed by the same letter do not differ significantly from one another ($P<0.05$).

The HMW-GS of the perennial material were fractionated by SDS-PAGE and classified according to the nomenclature described by Payne and Lawrence (1983) and Pogna *et al.* (1989) (Figure 1). Six perennial lines (11955, OK72, OT38, 235A, 280B and 281B) showed no trace of HMW-GS inherited from the wheatgrass parent and exhibited the commonly occurring subunits 1 or 2* encoded by the *Glu-A1* locus on the long arm of chromosome 1A together with subunits 20, 7*+ 8 or 7+9 encoded by the *Glu-B1* locus (chromosome 1BL) and subunit pairs 2+12 or 5+10 encoded by the *Glu-D1* locus (chromosome 1DL) (Table 4). By contrast, lines 236A, 244B and

251B exhibited unusual HMW-GS, likely inherited from the wheatgrass parent (Figure 1, arrows) and lacked HMW-GS encoded by the *Glu-D1* locus on chromosome 1DL. In addition, line 244B did not show any subunit encoded by the *Glu-B1* locus. SDS-PAGE fractionation of 10 single kernels from each genotype revealed that the perennial wheat derivatives were homogeneous for their HWM-GS patterns, with the only exception being line 281B, which turned out to be a mixture of three different genotypes (biotypes) with contrasting HMW-GS compositions at *Glu-A1* (subunit 1 or Null) and *Glu-D1* (subunit pair 2+12 or 5+10) (Figure 2 and Table 4).

FIGURE 1. SDS-PAGE FRACTIONATION OF TOTAL PROTEINS FROM NINE PERENNIAL WHEAT DERIVATIVES

(1) line 235A, (2) line 236A, (3) line 244B, (4) line 251B, (5) line 280B, (6) line 281B, (7) line 11955, (8) line OK72 and (9) line OT38. HMW-GS are numbered. Arrowheads indicate HMW-GS inherited from wheatgrass (*Thinopyrum* ssp).

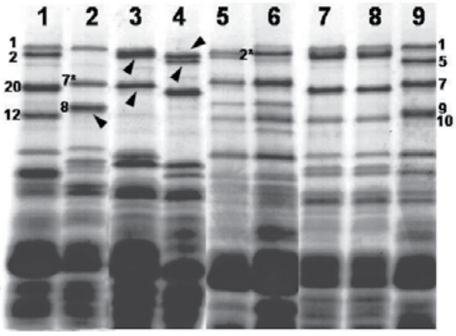


FIGURE 2. SDS-PAGE PATTERN OF TOTAL PROTEINS FROM THREE SINGLE SEEDS OF PERENNIAL WHEAT LINE 281B

HMW-GS are numbered.

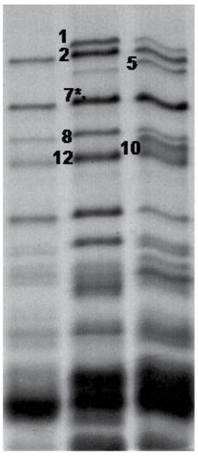


TABLE 4. HMW-GS COMPOSITION OF NINE PERENNIAL WHEAT DERIVATIVES

WHEAT GENOTYPE	WHEAT LOCUS			WHEATGRASS LOCUS
	GLU-A1	*GLU-B1*	*GLU-D1*	
11955	1	20	2+12	absent
OK72	1	20	2+12	absent
OT38	1	7+9	5+10	absent
235A	1	20	2+12	absent
236A	1	7+8	absent	1 subunit
244B	1	absent	absent	2 subunits
251B	Null	20	absent	2 subunits
280B	2*	7+8	2+12	absent
281B-1*	1	7+8	2+12	absent
281B-2	Null	7+8	2+12	absent
281B-3	1	7+8	5+10	absent

*Line 281B contains 3 biotypes with contrasting HMW-GS compositions

Gliadin patterns of the perennial wheat derivatives fractionated by A-PAGE were comparable with those of annual wheat cvs. Bolero and Chinese Spring. However, lines 236A, 244B, 251B and OT38 revealed some ω- or γ-gliadins inherited from the wheatgrass parent (Figure 3, arrowhead). Upon A-PAGE fractionation of gliadins from single seeds, line 236A (Figure 4), 235A and 281B turned out to be a mixture of two or more biotypes with contrasting gliadin bands encoded by homoeologous group 1 chromosomes of common wheat.

FIGURE 3. A-PAGE FRACTIONATION OF GLIADINS FROM NINE PERENNIAL WHEAT DERIVATIVES

(1) Line 235A, (2) line 236A, (3) common wheat cv. Enesco,(4) line 244B, (5) line 251B, (6) line 280B, (7) line 281B, (8) line 11955, (9) line OK72 and (10) line OT38. Arrowheads indicated gliadin inherited from wheatgrass (*Thinopyrum* spp.).

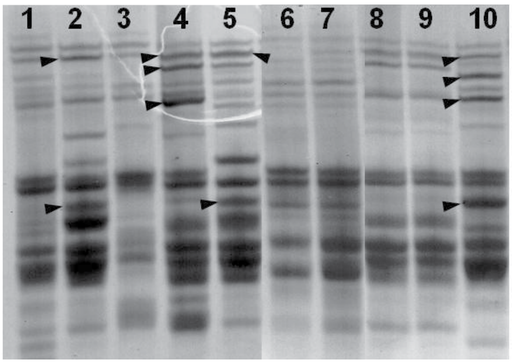
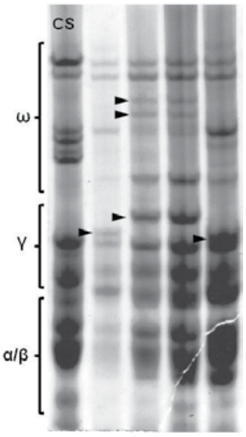


FIGURE 4. A-PAGE FRACTIONATION OF GLIADINS FROM COMMON WHEAT CV. CHINESE SPRING (CS) AND FOUR SINGLE SEEDS OF PERENNIAL WHEAT LINE 236A

Arrowheads indicate differential gliadin bands.



Kernel hardness was determined by the SKCS method using 50 grains for each line and found to be typical of soft-textured (mean SKCS index=30) or medium-hard common wheat (mean SKCS index =68) (Table 5).

TABLE 5. MEAN SKCS VALUE AND ALLELE COMPOSITION AT THE PUROINDOLINE LOCI IN NINE PERENNIAL WHEAT DERIVATIVES

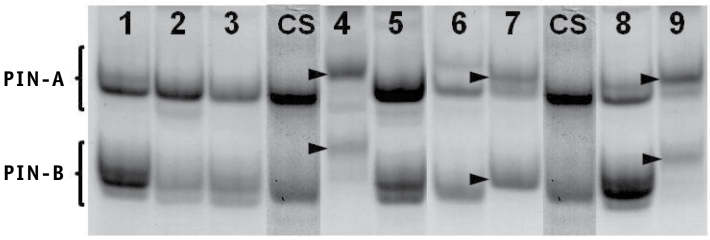
PERENNIAL WHEAT LINE	WHEAT LOCUS		WHEATGRASS LOCUS		SKCS
	PIN-A	*PIN-B*	*PIN-A*	*PIN-B*	
235A			Novel allele	Novel allele	55.4b
236A	*Pina-D1a*	*Pinb-D1a*			34.5c
244B	*Pina-D1a*	*Pinb-D1a*			46.7bc
251B	*Pina-D1a*	*Pinb-D1a*			35.4c
280B			FJ898232.1*	nd	69.1b
281B			FJ898232.1*	nd	60.0b
11955	*Pina-D1a*	*Pinb-D1a*			29.5c
OK72	*Pina-D1a*	*Pinb-D1a*			32.5c
OT38	*Pina-D1a*	nd			37.7c
Wedgetail	*Pina-D1a*	*Pinb-D1b*			61.5b
Enesco	*Pina-D1b*	*Pinb-D1a*			79.8a

nd, not determined ; * GenBank number. In the last column, means followed by the same letter do not differ significantly from one another ($P<0.05$).

Upon A-PAGE fractionation, the perennial wheat derivatives exhibited puroindoline-A (PIN-A) and puroindoline-B (PIN-B) inherited from either wheatgrass or common wheat. Novel, slow-moving PIN-A and PIN-B likely inherited from wheatgrass (*Thinopyrum spp*.) occurred in the medium-hard kernels produced by lines 235A, 280B and 281B (Figure 5, arrowheads). By contrast soft-textured perennial lines 236A, 244B, 251B, 11955, OK72 and OT38 exhibited wild-type PIN-A and PIN-B. When submitted to PCR amplification and sequencing, these latter soft lines revealed alleles *Pina-D1a* (coding for PIN-A) and *Pinb-D1a* (PIN-B) inherited from common wheat, whereas the medium-hard lines showed three unusual alleles (Table 5). In particular, the PIN-A allele in lines 280B and 281B was found to be identical to that amplified in *Aegilops tauschii* isolate TT52 (GenBank reference no. FJ898232.1), whereas the PIN-A and PIN-B alleles in line 235A were novel sequences never described before.

FIGURE 5. A-PAGE FRACTIONATION OF PUROINDOLINES A (PIN-A) AND B (PIN-B) IN WHEAT CV. CHINESE SPRING (CS) AND PERENNIAL WHEAT LINES (1) OK72, (2) 244B, (3) 251B, (4) 280B, (5) 236A, (6) OT38, (7) 235A, (8) 11955 AND (9) 281B

Arrowheads indicate puroindolines inherited from wheatgrass (*Thinopyrum* spp.).



Phytochemical profile of perennial wheat derivatives

Wholemeals from perennial wheat derivatives and cv. Wedgetail harvested in 2012 (first harvest) were compared for their content in resistant starch (RS), AR, soluble polyphenols (SP), and DF. The YP content of the representatives of perennial wheat varied in the ranges 5.12 to 11.37 ppm, their average content being 43 percent higher than that of cv. Wedgetail (Table 6).

TABLE 6. YELLOW PIGMENT (YP), DIETARY FIBRE (DF), 5-N-ALKYLRESORCINOLS (AR), SOLUBLE POLYPHENOLS (SP), TOTAL STARCH (TS), RESISTANT STARCH (RS) AND RS/TS RATIO IN 9 PERENNIAL WHEAT DERIVATIVES AND ANNUAL WHEAT CV. WEDGETAIL HARVESTED IN 2012 (FIRST HARVEST)

GENOTYPE	YP (PPM)	DF (%)	AR (µg/g)	SP (mg/g)	TS (%)	RS (%)	RS/TS (X100)
235A	5.1 e	15.9 bc	384 d	206 h	57.8 b	0.71 ab	1.2
236A	6.8 c	15.1 d	266 h	287 e	46.4 f	0.47ab	1.0
244B	6.0 d	16.9 a	329 g	231 g	49.9 e	0.74 a	1.5
251B	7.0 c	13.9 e	391 c	255 f	57.9 b	0.59 ab	1.0
280B	11.4 a	15.3 cd	500 b	640 a	57.4 bc	0.49 ab	0.9
281B	9.4 b	13.6 e	372 e	309 d	55.4 c	0.46 b	0.8
11955	6.9 c	16.3 ab	522 a	406 b	51.4 de	0.63 ab	1.2
OK72	6.2 d	16.3 ab	346 f	193 i	68.3 a	0.58 ab	0.8
OT38	7.1 c	12.8 f	182 i	340 c	53.3 d	0.45 b	0.9
MEAN	7.3±1.9	15.1±1.4	366±105	319±280	55.3±6.2	0.6±0.1	1.0±0.22
cv. Wedgetail	5.1	13.4	308	280	73.7	0.4	0.5

In each column, means followed by the same letter do not differ significantly from one another (P<0.05).

On average, the total content in 5-alkylresorcinol (AR), soluble polyphenols (SP) and DF was high in the perennial wheat derivatives compared with cv. Wedgetail. However, there was a considerable variability for these bioactive compounds among the germplasm in this study. For instance, the lowest and highest SP values of 193 mg and 600 mg were determined in lines OK72 and 280B, the range of variation among these lines being as high as 407 mg. Another example of variability includes lines OT38 and 11955, which showed 5-n-alkylresorcinol contents of 182 mg/g and 522 mg/g, respectively. As expected, the high protein content of the perennial wheat lines was associated with a reduced amount of total starch (TS) compared with the annual wheat control. Interestingly, all perennial wheat lines exhibited a high concentration of RS, which resulted in a high RS/TS ratio (Table 6). No significant correlation was found between seed weight and the amount of phytochemicals AR, SP, DF and RS.

GC-MS analysis was used to determine the alkylresorcinol homologue composition of grain harvested in 2012. Compared with common wheat cv. Wedgetail, perennial wheat lines 236A, OK72 and OT38 revealed an unusual AR pattern, with a prevalence (> 50 percent) of C19:0 homologue and a relatively high percentage (11-16 percent) of C17: 0 (Table 7). In addition, the nine perennial wheat representatives were found to belong to two groups based on the C17/C21 ratio, which is peculiar of the different *Triticum* species. The first group includes six lines with a C17/C21 ratio of 0.09 to 0.22, comparable to that of the control cv. Wedgetail (0.11), while the second group includes three lines (236A, OK72 and OT38) with a C17/C21 ratio of 0.34 to 0.76.

TABLE 7. HOMOLOGUE PROFILES (%) OF 5-N-ALKYLRESORCINOLS IN NINE PERENNIAL WHEAT DERIVATIVES

GENOTYPE	HOMOLOGUE						C17/C21 RATIO
	C15:0	C17:0	C19:0	C21:0	C23:0	C25:0	
235A	0.54±0.03	5.00±0.23	29.80±1.25	43.21±1.90	13.49±0.86	7.97±0.28	0.12
236A	0.21±0.13	12.61±1.63	50.89±0.50	31.77±1.69	3.98±0.22	0.54±0.24	0.40
244B	0.77±0.12	7.19±0.13	43.05±1.66	36.85±0.89	8.73±0.83	3.41±0.54	0.20
251B	0.46±0.19	6.52±0.63	39.62±1.65	40.66±2.13	8.72±0.19	4.02±0.24	0.16
280B	0.64±0.15	8.06±0.21	38.04±1.18	36.87±0.34	10.22±0.70	6.16±0.65	0.22
281B	0.51±0.19	4.21±0.22	30.72±1.09	47.80±1.27	11.67±0.32	5.09±0.85	0.09
11955	0.44±0.09	6.70±0.24	43.75±0.75	39.78±0.63	6.82±0.19	2.52±0.12	0.17
OK72	0.43±0.11	11.20±0.89	51.02±1.45	32.91±1.60	3.71±0.67	0.73±0.17	0.34
OT38	0.24±0.16	16.33±2.39	60.73±3.17	21.57±0.95	0.99±0.12	0.14±0.11	0.76
MEAN	0.47±0.16	8.65±0.99	43.07±1.35	36.82±1.11	7.59±0.23	3.40±0.25	
LSD (0.05)	0.38	1.20	1.40	1.25	0.78	0.57	
Cv. Wedgetail	0.68±0.31	4.84±0.26	36.72±0.33	45.23±0.27	9.22±0.19	3.30±0.04	0.11

DISCUSSION

Agronomic traits and PHR

The significant differences in the mean PHR value and the wide variation of this trait (5.6 to 42.0 percent) in the nine perennial wheat derivatives developed by the Land Institute and Washington State University indicate that several genes interacting with environment and climatic conditions play an important role in modulating regrowth after harvest. Survival of regrowing plants through the 2012 winter following the first harvest was very high, approaching 100 percent in most lines and leading to a small decline of PHR in 2013. This was likely due to the mild temperatures registered during autumn 2012-winter 2013 in the Tiber valley. The mean PHR scores of 27.8 percent in 2012 and 25.9 percent in 2013 and the performance of lines OK72, 251B, 281B and 11955 which showed regrowth scores greater than 33 percent indicates that the present germplasm is valuable for its regrowth potential. These results confirm the recent conclusions by Hayes *et al.* (2012), who pointed out the strong relationship between PHR and the presence of at least one whole genome equivalent of 14 chromosomes from the wheatgrass parent. According to these authors, lines with $2n = 56$ chromosomes in Table 1 likely contain 42 wheat chromosomes plus 14 wheatgrass chromosomes. However, wheat derivatives with a relatively high regrowth score may contain a reduced number of chromosomes due to substitution of one or more wheat chromosomes by wheatgrass homoeologous (Hayes *et al.* 2012). Here, evidence has been obtained that substitution or recombination involving E-genome and ABD-genome chromosomes likely occurred in lines 236A, 244B and 251B, which have $2n$=56 and *Th. elongatum* in their pedigree. In addition, all perennial wheat derivatives showed endosperm proteins inherited from either wheat or wheatgrass parents. These aspects will be discussed later. The current study identified some negative agronomic attributes, mainly tenacious glumes and reduced kernel weight, together with many desirable traits such as reduced plant height, high tiller number and disease resistance, which can contribute to increase the agronomic potential of perennial wheat.

Quality traits

The HMW-GS account for only about 1 percent of the dry weight of wheat kernel (Payne *et al.* 1987) Nevertheless the results presented in this paper are consistent with accumulated evidence that they are the principal subunits that impart elasticity to gluten. Variation in composition of HMW-GS among the perennial material was found to make a large contribution to the gluten quality of these genotypes. In particular, the absence of HMW-GS encoded by the *Glu-D1* locus on the long arm of chromosome 1D in lines 236A, 244B and 251B (Figure 1, lanes 2-4) proved to be deleterious to gluten quality as determined by the SDS sedimentation volume (Table 3). In these lines, the presence of HMW-GS inherited from wheatgrass (Figure 1, arrowheads) could additionally affect gluten quality. Furthermore, lines 236A, 244B and 251B contain 1-3 w-gliadins plus one prominent g-gliadin arrowed in Figure 3, which have been likely inherited from their wheatgrass parent. In common wheat, all ω-gliadins and most γ-gliadins are encoded by genes on the short arms the homoeologous group 1 chromosomes (Payne *et al.* 1984). Moreover, a comparative study showed that all species in the genera *Triticum*, *Aegilops*, *Secale* and *Hordeum* contain genes coding for prolamins HMW-GS, gliadins or gliadin-type proteins such as secalins and hordeins) on homoeologous chromosome 1, suggesting that wheatgrass prolamins in lines 236A, 244B and 251B are likely encoded by chromosome 1E from *Thinopyrum elongatum*. As introgression of wheatgrass prolamin genes into perennial wheat lines 236A, 244B and 251B has been accompanied by concomitant removal of the *Glu-D1* locus, it is not clear whether it is the presence of wheatgrass prolamins or the absence of wheat HMW-GS that negatively impact the bread-making quality. Line OT30 could offer an opportunity to elucidate this aspect. According to the quality score assigned to each HMW subunit or subunit pair based on its effect on gluten quality (Payne *et al.* 1987), the HMW-GS composition of line OT38 (subunit 1 of chromosome 1A, subunits 7+9 of 1B and subunits 5+10 of 1D, Table 4) has the high *Glu-1* quality score of nine, the maximum score being ten and the minimum three. The finding that the presence of wheatgrass gliadins in line OT38 (Figure 10, arrowheads) is associated with a SDS sedimentation volume as low as 47 ml (Table 3) suggests a direct negative effect of these proteins on gluten quality. In this context it is worth noting that some European-grown wheat cultivars contain the short arm of chromosome 1R from rye combined with the long arm of chromosome 1B (1BL/1RS). This translocated chromosome causes a decrease in gluten quality due to the presence of 1RS-encoded secalins, which increases dough stickiness (Zeller *et al.* 1982). The negative effects of rye prolamins on bread making quality of hexaploid AABBRR triticale are documented as well, and substitution of chromosome 1D for chromosome 1R dramatically improved such parameters of bread-making quality of triticale as SDS-sedimentation, mixing time, mixing tolerance, and loaf volume (Kazman and Lelley, 1996).

Kernel texture, a major determinant of flour quality and end-use quality of wheat, is mainly modulated by allele variation at the *Pina-D1* and *Pinb-D1* loci on chromosome 5DS coding for PIN-A PIN-B, respectively. In the present work, the molecular analysis of puroindoline genes from perennial wheat lines of different genetic origins identified novel alleles coding for PIN-A and PIN-B in line 235A with *Th. elongatum* in the pedigree. When compared with wild-type *Pinb-D1a* allele, the gene coding for PIN-B in this line shows 19 SNPs, whereas the encoded PIN-B protein contains nine amino acid substitutions in its mature form. The novel *Pina-E1a* and *Pinb-E1a* alleles in the homoeologous chromosome 5ES inherited from of *Th. elongatum* conferred a medium hard texture to line 235A (SKCS value = 55.4, Table 5). Interestingly, the absence of *Pina-D1* and *Pinb-D1* sequences from chromosome 5DS suggests that 5E (5D) chromosome substitution or homoeologous recombination between wheatgrass chromosome 5E and 5D of wheat occurred in line 235A.

Medium-hard kernel texture in lines 280B and 281B was associated with the presence of two unusual puroindolines with reduced mobility with respect to wild-tipe PIN-A and PIN-B (Figure 5, lanes 4 and 9). As *T. carthlicum* does not contain *Pina* and *Pinb* genes, puroindolines in lines 280B and 281B have been likely inherited from *Th. intermedium*. The 100 percent similarity between the DNA sequence coding for PIN-A in lines 280B and 281B and the *Pina-D1* allele in *Aegilops tauschii* isolate TT52 (GenBank reference no. FJ898232.1) suggests a strong phylogenetic relationship between D genome and J, J^S or S genome of *Th. intermedium*. As observed in line 235A, the absence of *Pina-D1* and *Pinb-D1* sequences from chromosome 5DS as determined by PCR amplification suggests chromosome substitution or allosyndetic recombination involving chromosome 5D of wheat and a homoeologous chromosome of *Th. intermedium*.

Nutritional traits

Based on the genotypes studied here, there is a great variation in the phytochemical composition among wheat cv. Wedgetail and the nine representatives of perennial wheat. In particular, there is an increased amount of yellow pigments, dietary fibre and RS in most of the perennial wheat derivatives analysed. In addition, unique bioactive phytochemical patterns with high levels of both 5-alkylresorcinol (AR) and soluble polyphenols (SP) were detected in lines 280B and 11955. Among the nine perennial wheat genotypes, line 11955 is characterized by relatively high values for kernel weight (30.1 mg), number of kernels/spike and PHR (33.3 percent). In addition, line 11955 showed the *Glu-1* quality score of six coupled with an above-average SDS sedimentation volume of 50 ml, suggesting its use as a component of a range of traditional and specialty products naturally enriched with health-promoting compounds.

The C17:0/C21:0 ratio of AR homologues has been used to distinguish between different cereal species. This ratio ranges between 0.01 in *Triticum monococcum*, 0.02 in *Triticum turgidum* ssp *dicoccum*, 0.05 in T. *turgidum* ssp *durum*, 0.06 in *T. timopheevii*, 0.11 to 0.18 in common wheat and *T. turgidum* ssp *turanicum,* and 0.25 in *T. zhukovskyi* (Ross *et al.* 2003; Ciccoritti *et al.* 2013). On average, the nine perennial wheats showed a high proportion of C17:0 and a low proportion of C21:0 homologues compared with cv. Wedgetail. This was mainly due to lines OT38, 236A and OK72, which were unique in having C17/C21 ratios as high as 0.34 to 0.76 (Table 7). By contrast, the remaining six perennial lines exhibited C17/C21 ratios of 0.09 to 0.22 comparable to those observed in the different species of the genus *Triticum*. Interestingly, lines 235A and 281 showed unusual AR homologue compositions with a high proportion of C21:0, C23:0 and C25:0 homologues (complessively about 64.5 percent as compared with 55.8 percent in cv. Wedgetail). As long-chain resorcinolic lipids affect protein structure and activity (Stasiuk *et al.* 2008), these lines may have some potential as a source of cereal foods for prevention of cardiovascular diseases and cancer.

The successful development of perennial wheat cultivars and their widespread adoption by millers, bakers and consumers will be facilitated by improvement of kernel threshability, milling and bread making quality, and nutritional characteristics including gluten digestibility (tolerance). Therefore, in addition to addressing the major agronomic traits (grain yield, PHR and disease resistance), good milling and baking quality and superior nutritional quality seem to be key traits to target for genetic improvement. The wide variation in storage protein composition and bioactive compounds detected in the germplasm analysed here can be easily exploited by breeders in the development of new perennial wheat genotypes with improved end-use qualities.

REFERENCES

AOAC, 1975. *Pigment in flour*. Official Methods of Analysis, 12th edition, Washington D.C.,USA.

AOAC, 1995. *Insoluble dietary fiber in foods-enzymatic gravimetric method*. Official Methods of Analysis, 16th edition, Washington D.C.,USA.

Bell, L.W., Byrne, F., Ewing, M.A. & Wade, L.J. 2008. A preliminary whole-farm economic analysis of perennial wheat in an Australian dryland farming system. *Agricultural Systems.* 96: 166-174.

Bellato, S., Ciccoritti, R., Del Frate V., Sgrulletta D., Carbone K. 2013. Influence of genotype and environment on the content of 5-n-alkylresorcinols, total phenols and on the antiradical activity of whole durum wheat grains. *Journal of Cereal Science*. 57: 162-189

Ciccoritti, R., Carbone, K., Bellato, S., Pogna, N. & Sgrulletta, D. 2013. Content and relative composition of some phytochemicals in diploid, tetraploid and hexaploid *Triticum* species with potential nutraceutical properties. *Journal of Cereal Science.* 57: 200-206

Corona, V., Gazza, L., Boggini, G. & Pogna, N.E. 2001. Variation in friabilin composition as determined by A-PAGE fractionation and PCR amplification, and its relationship to grain hardness in bread wheat. *Journal of Cereal Science*. 34: 243 – 250.

Cox, T.S., Van Tassel, D.L., Cox, C.M. & DeHaan, L.R. 2010. Progress in breeding perennial grains. *Crop Pasture Science*. 61: 513-521

Crews, T.E. 2005. Perennial crops and endogenous nutrient supplies. *Renewable Agriculture and Food Systems.* 20: 25-37.

DeHaan, L.R., Van Tassel, D.L. & Cox, T.S. 2005. Perennial grain crops: A synthesis of ecology and plant breeding. *Renewable Agriculture and Food Systems.* 20:5-14.

DeWet, J.M. 1981. Grasses and the culture history of man. *Annals of Missouri Botanical Garden.* 68: 87-104.

Gautier, M.F., Aleman, M.E., Guirao, A., Marion, D. & Joudrier, P. 1994. *Triticum aestivum* puroindolines, two basic cysteine-rich seed proteins: cDNA analysis and developmental gene expression. *Plant Molecular Biology.* 25:43-57.

Glover, J.D. 2005. The necessity and possibility of perennial grain production systems. *Renewable Agriculture and Food Systems.* 20: 1-2.

Hayes, R.C., Newell, M.T., DeHann, L.R., Murphy, K.M., Crane, S., Norton, M.R., Wade, L.J., Newberry, M., Fahim, M., Jones, S.S., Cox, T.S. & Larkin, P.J. 2012. Perennial cereal crops : An initial evaluation of wheat derivatives. *Field Crop Research.* 133:68-89.

Kazman, E.M. & Lelley, T. 1996. Can breadmaking quality be introduced into hexaploid triticale by whole chromosome manipulation? In: H. Guedes-Pinto *et al.* (ed.) *Triticale Today and Tomorrow*, Kluwer Academic Publishers, Dordrecht, The Netherlands.Koebner, p 141–148.

Jordan N, Boody G, Broussard W, Glover JD, Keeney D, McCown BH, McIsaac G, Muller M, Murray H, Neal J, Pansing C, Turner E, Warner K, Wyse D, 2007. Environment – sustainable development of agricultural bio-economy. *Science.* 316: 1570-1571

Landberg, R., Andersson, A.A.M., Aman, P. & Kamal-Eldin, A., 2009. Comparison of GC and colorimetry for the determination of alkylresorcinol homologues in cereal grains and products. *Food Chemistry.* 113: 1363-1369.

Li, S., Morris, C.F. & Bettge, A.D. 2009. Genotype and environment variation for arabinoxylan in hardwinter and spring wheats of the U.S. Pacific Northwest. *Cereal Chemistry* 86: 88–95.

McCleary, B. V., Gibson, T. S. & Mugford, D. C. 1997. Measurement of total starch in cereal products by amyloglucosidase-α-amylase method: Collaborative study. *Journal of AOAC International.* 80: 571-579.

McCleary, B.V. & Monaghan, D.A. 2002. Measurement of resistant starch. *Journal of AOAC International* 85: 665-675

Mc Cleary B.V., Mc Nally M. and Rossiter P. 2002. Measurement of resistant starch by enzymatic digestion and selected plant materials: collaborative study. *Journal of AOAC International.* 85: 1103.

Moore, J. & Yu, L.L. 2008. Methods for antioxidant capacity estimation of wheat and wheat-based food products. In: Yu, L. (Ed.), *Wheat Antioxidant*. Mac Graw-Hill, New York, pp. 147-150.

Murphy, K.M., Lyon, S.R., Balow, K.A. & Jones, S.S. 2010. Post-sexual cycle regrowth and grain yield of *Thinopyrum elongatuom* x *Triticum aestivum* amphiploids. *Plant Breeding.* 129: 480-483.

Payne, P.I., Holt, L.M., Jackson, E.A. & Law, C.N. 1984. Wheat storage proteins: Their genetics and their potential for manipulationby plant breeding. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 304: 359–371.

Payne, P.I. & Lawrence, G.J. 1983. Catalogue of alleles for the complex gene loci, *Glu-A1*, *Glu-B1* and *Glu-D1* which code for high molecular-weight subunits of glutenin in hexaploid wheat. *Cereal Research Communications.* 11: 29-35.

Payne, P.I., Nightingale, M.A., Krattiger, A.F. & Holt, L.M. 1987, The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. *Journal of Science and Food Agriculture.* 40: 51-65.

Pimentel, D., Harvey, C., Resosudarmo, P., Sinclair, K., Kurz, D., McNair, M., Crist, S., Shpritz, L., Fitton, L., Saffouri, R. & Blair, R. 1995. Environmental and economic costs of soil erosion and conservation benefits. *Science.* 267:1117-1123.

Pogna, N.E., Autran, J.C., Mellini, F., Lafiandra, D., & Feillet, P. 1990. Chromosome 1B-encoded gliadins and glutenin subunits in durum wheat: Genetics and relationship to gluten strength. *Journal of Cereal Science*. 11:15–34.

Pogna, N.E., Mellini, F., Beretta, A. & Dal Belin Peruffo, A. 1989. The high-molecular-weight glutenin subunits of common wheat cultivars grown in Italy. *Journal of Genetics & Breeding.* 43: 17-24.

Reganold, J.P., Elliot, L.F. & Unger, Y.L. 1987. Long-term effects of organic and conventional farming on soil erosion. *Nature.* 330:370-372.

Ross, A., Shepherd, M., Schupphaus, M., Sinclair, V., Alfaro, B., Kamal-Eldin, A. & Aman, P. 2003. Alkylresorcinols in cereals and cereal products. *Journal of Agricultural Food Chemistry.* 51: 4111-4118.

Stasiuk, M., Bartosiewicz, D. & Kozubek, A. 2008. Inhibitory effect of some natural and semisynthetic phenolic lipids upon acetylcholinesterase activity. *Food Chemistry.* 108: 996-1001.

Tsintin, N.V. & Lubinova, V.F. 1959. New species and form of cereals derived from hybridization between wheat and couch grass. *American Naturalist*. 93: 181-191.

Wagoner, P. 1990. Perennial grain development: past efforts and potential for the future. *Critical Reviews in Plant Sciences.* 9(5):381-408.

Wang, Y.G., Khan, K., Hareland, G. & Nygard, G. 2007. Distribution of protein composition in bread wheat flour mill streams and relationship to breadmaking quality. *Cereal Chemistry.* 84: 271-275.

Zeller, F.J., Gunzel, G., Fischbeck, G., Gerstenkorn, P. & Weipert, D. 1982. Veranderung deBackeigenschaften der Weizen-Roggen-Chromosomen-Translokation 1B/1R. *Getreide Mehl Brot.* 36:141–143.

06

CURRENT EFFORTS TO DEVELOP PERENNIAL WHEAT AND DOMESTICATE *THINOPYRUM INTERMEDIUM* AS A PERENNIAL GRAIN

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ABSTRACT

We are developing a new perennial grain by domesticating the perennial grass *Thinopyrum intermedium* (intermediate wheatgrass). In 1983, intermediate wheatgrass was selected for domestication by the Rodale Institute (Kutztown, Penn., USA). Nearly 100 species of perennial grasses were evaluated for promise as a perennial grain before choosing intermediate wheatgrass to domesticate. The Rodale Institute performed two cycles of selection, beginning in 1988. Using selections made by Rodale, breeding work began at The Land Institute (Salina, Kan., USA) in 2002.

Selection has been for yield per head, increased seed mass, free threshing ability, reduced height, and early maturity. Two cycles of selection at The Land Institute have increased seed yield by about 77 percent and seed mass by about 23 percent, when grown in a solid stand. Selected materials have been found to possess a higher harvest index and reduced plant spread.

Molecular tools are being developed for intermediate wheatgrass. A combination of expressed sequence tag (EST), SSRs and AFLP markers will be used to genotype an experimental mapping population comprises 268 full-sib progeny derived from a reciprocal cross of two experimental genotypes. Genotyping by sequencing is also being used to identify ~3 000 high quality single-nucleotide polymorphisms (SNPs) in a population derived from one selfed individual. Phenotype data has been collected and will be used to identify QTL associated with SNPs. Using gene cloning, protein separation and identification, and sequence alignments; we were able to identify five HMW-GS genes and their allelic variants in intermediate wheatgrass plants.

Since 2001, we have been working to develop perennial wheat by crossing wheat (*Triticum* spp.) with perennial *Thinopyrum* species. We have obtained a few stable lines with one set (12-14) *Thinopyrum* chromosomes and 42 wheat chromosomes. These have better agronomic performance in Kansas than other materials, but lack perenniality. Crosses between winter durum wheat and *Thinopyrum intermedium* have been recently developed, and they are promising in terms of perenniality, seed weight, winter hardiness, and vigor. To study the impact of annual/perennial genome dosage on perenniality and agronomic performance, we have crossed diploid and tetraploid wheat lines with tetraploid and hexaploid *Thinopyrum* species. All F_1 plants are perennial, and many have been doubled with colchicine. Wheat chromosome-specific markers have been used to characterize 94 plants that were perennial in the field. Plants with more wheat chromosomes eliminated tended to be more perennial, but specific chromosomes were not associated with perenniality or annuality.

INTRODUCTION

Two major approaches are being used to develop perennial small grains: wide hybridization and domestication. The two approaches present unique strengths and challenges. Wide hybridization involves crossing an annual grain such as wheat with related perennial species. Wide crosses will in theory make available genes controlling traits such as yield, seed size, free threshing ability, and quality, which have been accumulated in current grain crops. With wheat the challenge has been to obtain cytogenetic stability in wide hybrids while preserving perenniality and domestication traits. Direct domestication of wild perennials has the potential benefit of working with populations that are vigorous perennials. However, the necessary genetic variation for domestication may be lacking in perennial species, or substantial time may be required for selection to achieve adequate seed size, yield, or other domestic traits.

The first sustained effort to directly domesticate a perennial grass for grain, of which we are aware, was initiated by researchers at The Rodale Institute in Pennsylvania, United States of America (Wagoner, 1990). The researchers leading this domestication effort cite Jackson (1980) as the inspiration for developing perennial grasses for grain. In 1983, researchers began to evaluate nearly 100 perennial grasses to determine their suitability for grain production. Based on numerous criteria, intermediate wheatgrass (*Thinopyrum intermedium*) was selected as a perennial grain candidate. Beginning in 1988, two cycles of selection for improved fertility, seed size, and other traits were performed in Pennsylvania.

Selection for grain production in intermediate wheatgrass was continued by workers at The Land Institute in Kansas, United States of America, beginning in 2003 (Cox *et al.* 2010). Four cycles of selection using an index based primarily on seed yield per head, seed mass, and free threshing ability have been performed at The Land Institute. Improved populations resulting from this selection program are currently being evaluated and further selected by collaborators in diverse environments.

We began a perennial wheat (*Agrotriticum*) breeding program in 2001. Perennial wheat has potential in reducing soil erosion and promoting the sustainability of agriculture (Cox *et al.* 2002; Cox *et al.* 2006). Through perennial wheat, or wheat-*Thinopyrum* hybrids, more than 18 agronomically important traits other than perenniality have been transferred from *Thinopyrum* species into wheat (Chen *et al.* 2005). At the beginning of our breeding program, we introduced perennial wheat lines from other institutions. All of them died at a time point after grain harvest during the hot summer of Kansas (Cox *et al.* 2006). Therefore, we have sought fresh approaches to developing truly perennial wheat. Among these new approaches are management techniques. Because we have yet to identify agronomic strategies to induce perenniality in Kansas, here we will focus on genetic approaches.

RECENT PROGRESS

Breeding Perennial Wheat

In our search for an approach to perennial wheat with good grain yield and perenniality, we have crossed wheat with *Th. ponticum* (10x = 70), *Th. intermedium* (6x = 42), and *Th. elongatum* (2x = 14). We have also attempted crosses with other *Thinopyrum* species. Crosses involving *Th. junceiforme* (4x = 28), *Th. bessarabicum* (2x = 14), and a *Th. bessarabicum-Th. elongatum* hybrid (4x = 28) have produced hybrid F_1 plants successfully. The crosses with *Th. junceum* (6x = 42) and *Th. scerpium* (4x = 28) could form well-developed embryos. But the plantlets died shortly after germination on nutrient medium. One *Thinopyrum* accession (6x = 42; PI531731) is unique in that it is strictly self-pollinated. We have attempted to cross 6x and 4x wheat with this accession. However, no hybrid embryos have been obtained as the caryopses aborted at early stage. We have successfully obtained F_1 hybrids between *Thinopyrum* species and both rye and triticale. So far, no crosses involving rye have resulted in lines with perenniality and seed fertility.

In recent years, we have created a couple of genetically stable lines which were derived from the crosses between common wheat (*T. aestivum*) and *Th. intermedium* wheatgrass. Similar to a great number of perennial wheat lines developed by other institutions, these lines had chromosome numbers from 54 to 56 with 12 to14 chromosomes from wheatgrass. Minor translocations involving wheatgrass chromosomes were observed on some wheat chromosomes. In our plot experiments, four breeding lines named B373, B1126#1, B1126#2 and B1321 did much better than the others. They looked similar to annual wheat cultivars, ripened early, and had excellent seed fertility and better-filled grain. Interestingly, these four lines shared a common parent, B373. They were similar to B373 in many aspects morphologically, but had improved regrowth ability. The line B373 was just a few days later in maturity than local common wheat cultivars. The wheat cultivar Jagger, a leading wheat cultivar in Kansas from the 1990s through the 2000s, was the donor of wheat chromosomes to B373. 'Jagger' might have contributed to the improved adaptability of these lines. By contrast, perennial wheat lines from the former Soviet Union and Washington State did not present similar adaptability. They flowered extremely late, produced fewer heads, and filled grain poorly.

In a root-tube experiment, we compared the three "perennial" wheat lines B373, B1126#2, and OK7211542 with wheat cultivar Jagger and a *Th. intermedium* selection C3-2627. The total biomass, root biomass, shoot biomass, and grain weight of the three perennial wheat lines are intermediate to the wheat and wheatgrass controls, respectively, except that OK7211542 had larger total biomass and shoot biomass than both controls. Like perennial wheatgrass, perennial wheat lines showed larger root mass than the wheat cultivar, especially at depth. At maturity, part of their leaves and stems were still green. These differences appear to be associated with maturity time and the strength of perenniality.

The regrowth ability of our breeding lines varies widely in different years or environments, which was also observed in investigations by other researchers (Tsitsin, 1978; Murphy *et al.* 2009; Hayes *et al.* 2011; Jaikumar *et al.* 2012). Extremely hot, cold, and dry weather conditions all can suppress the regrowth or cause the death of new tillers. We noticed that irrigation or rain after anthesis could promote regrowth. We have kept a number of lines in the greenhouse for continuous observation. The plants were watered every day, so that they were not stressed by moisture. However, the plants died with no more than three grain harvests. The number of tillers declined over life cycles. Unlike wheatgrass plants, the position in the crown where new tillers originated moved upward over time, which could make buds and new tillers vulnerable to environmental stresses. Furthermore, the new tillers entered the reproductive stage quickly without the need of vernalization. We have not found a line among our materials showing a typical winter-type regrowth profile in which the new tillers stay dormant like wheatgrass. Tsitsin (1978) suggested that winter-type regrowth was desirable for stronger perenniality. Common to these tested lines is that they carried about one set of 14 wheatgrass chromosomes.

An exception is MT-2, which was selected for forage production by Montana State University. In the Kansas environment, this hybrid line among all those we have obtained from other institutions is the most perennial. This line has lived in our field for two years up to present. It roughly contains two wheat genomes (mean = 26.2 wheat chromosomes) and two wheatgrass genomes (mean = 29.2 wheatgrass chromosomes) and is genetically unstable (Jones *et al*. 1999). The instability of this durum wheat (*T. turgidum* ssp. *durum*) hybrid and similar durum derivatives has led some to question the feasibility of durum as a parent of perennial wheat.

The problem of genetic instability may possibly be overcome by taking advantage of genetic variability within tetraploid species. Among the progenies derived from a cross of durum wheat with *Th. junceiforme*, we have found lines likely to be stable, predicted on the basis of seed fertility. Tetraploid wheat species may also possess genes that could promote genetic stability. It seems unnecessary to backcross with common wheat as advised by Ellneskog-Staam and Merker (2002).

By means of backcrossing to wheatgrass or intermating different perennial hybrid progenies at their early generations, we obtained 843 truly perennial plants that survived the hot Kansas summer in 2010 for the first time. These plants varied greatly in perenniality, seed fertility, vigour, regrowth habit, and other traits. Using the genomic *in situ* hybridization (GISH) technique, we examined the genomic constitutions of 11 plants. They possessed chromosome numbers ranging from 53 to 70, with some wheat chromosomes lost. In order to identify which wheat chromosomes might be associated with the elevated perenniality, we screened 154 wheat chromosome-specific markers (http://probes.pw.usda.gov:8080/snpworld/Search↱) on a panel of wheat and wheatgrass parents. We chose these markers because they had been assigned to 21 wheat chromosomes individually and had the capacity to distinguish homologous chromosomes. The presence of a marker clearly indicated the presence of a wheat chromosome. Fifty-six markers that amplified only in wheat were selected eventually to genotype a subset of 94 perennial plants (at F_2 to F_5 generations). The results showed that the patterns of wheat chromosome elimination differed by plants. All 94 plants had lost more than one wheat chromosome. With more wheat chromosomes eliminated, a plant tended to be more perennial. However, we did not see a specific wheat chromosome that was missing consistently across all plants, which would have indicated that a single wheat chromosome had a deciding impact on perenniality. It might be concluded that decreased abundance of wheat chromosomes is necessary for perennial wheat to live longer in the Kansas environment. Alternatively, the ratio of annual to perennial chromosomes may be critical to perenniality.

Cox *et al.* (2006) proposed that more than one wheatgrass genome would be required for perennial wheat to live in the field for many years. With the increased abundance of wheatgrass chromosomes and decrease in wheat chromosomes, some new issues need to be addressed. For instance, at present we do not know what percentage of wheatgrass chromosomes is appropriate for stronger perenniality that will ensure survival for multiple years. We also do not know if the decrease in wheat chromosomes will lead to more severe genetic instability. When polyploid wheatgrass species are used in crosses, wheatgrass chromosomes tend to be eliminated gradually in subsequent generations until a stable genomic constitution is reached (Tsitsin, 1978). It is impossible to assess the effects of complete genomes adequately using advanced generation materials due to chromosome elimination. To investigate these issues, we are establishing a panel of full amphiploids with different genomic constitutions that are composed of wheat: wheatgrass chromosome ratios of 3:3, 3:2, 2:3, 2:2, 1:3, and 1:2, respectively. Diploid, tetraploid, and hexaploid wheat species have been crossed with tetraploid and hexaploid wheatgrass species successfully. A fraction of such full amphiploids have been chromosome doubled, including the F_1 hybrid (2n = 84) between common wheat and *Th. intermedium* wheatgrass. Comparisons among them will help us understand how many wheat and wheatgrass genomes will enable perenniality across diverse environments.

The role of cytoplasm in the crosses of wheat with wheatgrass species may be worth exploring. Wide hybridizations in wheat and oilseeds have indicated that sequence deletion was different in reciprocal crosses, reflecting the interaction between nucleus and cytoplasm (Gill, 1991; Song *et al.* 1995; Ma and Gustafson, 2008; Ozkan and Feldman, 2009). To the best of our knowledge, all the existing perennial wheat lines bear wheat cytoplasm. We speculate that due to incompatibility with wheat cytoplasm, some wheatgrass chromosomes or genes important to perenniality are preferentially eliminated or silenced. This may be why all existing lines are not as persistent as the F_1 hybrids or perennial parents. Viable embryos are very difficult to obtain when wheatgrass is used as the female parent. Out of about 120 heads pollinated, we obtained a single plant with cytoplasm from *Th. intermedium*. We are investigating whether wheatgrass cytoplasm will aid the retention of wheatgrass chromosomes important to perenniality.

The easy crossability of wheat with *Thinopyrum* species provides opportunities for the exchange of genetic information between annual and perennial grass species. Both genera have their own pros and cons. We regard perennial wheat a hybrid species that bridges two genera. Putting more wheatgrass chromosomes/genes into wheat or vice versa appears to be the approach to 'truly' perennial wheat. As indicated by MT-2 and our recent investigations, the cross of durum wheat and *Th. intermedium* might be the best approach to explore in the coming years.

Perennial wheat breeding has been conducted for over 80 years. Hundreds of perennial wheat lines have been developed by different institutions. Certainly survival is dependent upon environmental variables, and agronomic practices developed specifically for perennial wheat may be critical to the persistence of perennial wheat. But declining stands in even the most moderate environments leads us to conclude that genetic improvement will be essential to persistence. The lack of sufficient persistence remains the biggest problem in the development of perennial wheat. The existing lines (with one set of wheatgrass chromosomes) have partly inherited the traits (e.g. large roots, tolerance to abiotic and biotic stresses) that make perennial wheatgrass persistent across varied environments. Before we have 'truly' perennial wheat, the use of these traits could yield some agronomic and ecological benefits for the time being. For instance, recent study of hybrid lines revealed novel disease resistance (Turner *et al*. 2013). Although current lines lack perenniality, their grain traits are similar to wheat. Therefore, these types could be economically viable in the near term while reliably perennial wheat varieties are developed.

Intermediate Wheatgrass Breeding in Kansas

Selection at the Land Institute has been based on individual plants spaced 0.91 metres apart. The first selection cycle comprises 1 000 clones replicated three times. The second selection cycle used about 4 000 individual plants. After two cycles of selection primarily for seed yield per head and seed mass, the resulting populations were planted in a replicated solid-seeded trial at two locations. For controls, the base population (consisting of seed obtained from the Rodale Institute) and several forage varieties were used. For simplicity we are presenting data from the irrigated location in the second year of production.

Two cycles of selection resulted in a 77 percent increase in seed yield (Table 1). If progress were to continue in a linear manner, an additional 12 cycles of selection would result in yields of approximately 2 500 kg ha^{-1}, similar to annual wheat in Kansas. Since this evaluation was initiated, an additional two cycles of selection have been performed, requiring two years per cycle. Therefore, we expect that with sustained efforts yields in Kansas may be similar to wheat within 20 years.

Seed size has responded weakly to index selection in space plants, when evaluated in a solid stand (Table 1). If trends from the first two cycles continue, about 110 years of sustained effort would be necessary to achieve a seed size of 30 mg seed^{-1}, similar to annual wheat. Therefore, large seed size may only be attained by using other approaches. We are currently beginning efforts to introgress genes controlling seed size from wheat. Also, genomic approaches may be useful in screening diverse collections for alleles conferring increased seed size.

TABLE 1. PERFORMANCE OF INTERMEDIATE WHEATGRASS POPULATIONS BEFORE (CYCLE 0) AND FOLLOWING ONE (CYCLE 1) AND TWO (CYCLE 2) CYCLES OF SELECTION

Three intermediate wheatgrass cultivars (Luna, Manska, and Rush) and one tall wheatgrass cultivar (Jose) are included as controls.

	SEED YIELD	BIOMASS YIELD	SEED MASS	HARVEST INDEX	CP[a]	NDF[a]	ADF[a]
	$g\ m^{-2}$		$mg\ seed^{-1}$	%	$g\ Kg^{-1}$		
Cycle 0	39.2	1 770	3.92	2.21	37.4	687	465.3
Cycle 1	56.5	1 920	4.48	2.94	30.6	680	461.1
Cycle 2	69.3	1 740	4.84	3.93	33.5	662	444.5
Luna	17.8	1 480	4.10	1.22	45.0	630	447.5
Manska	21.6	1 610	3.41	1.35	37.0	653	435.7
Rush	26.3	1 730	3.61	1.55	34.5	655	440.6
Jose	6.6	1 500	4.06	0.46	44.7	683	478.7
SEM[b]	7.4	120	0.15	0.39	3.0	11	8.2

a Crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined for the non-seed biomass collected at seed maturity.

b Standard error of the mean.

Biomass production has remained mostly unchanged during selection for seed traits (Table 1). Therefore, rising grain yields are apparently occurring due to increased harvest index. In fact, across the populations evaluated the correlation between grain yield and harvest index was 0.996.

Forage quality indicators of the biomass residue were measured to determine whether selection for seed production was reducing forage quality (Table 1). So far the effects of selection have been minor relative to forage varieties, at least when measured at the late stage when seed is ripe. However, we expect that increased allocation of nitrogen to seed will ultimately reduce protein content of the residue. For systems involving both grain and forage production, selection for forage quality at seed maturity may be necessary.

During the third cycle of selection in space-planted nurseries, several individuals with outstanding yield per head were identified. A second set of plants with large seed size were also selected. These two groups of plants were cloned by dividing their crowns and used to establish bordered plots 0.91 metres square at two locations: Kansas and Minnesota. The Kansas location has higher summer temperatures, but was irrigated. The Minnesota location was not irrigated. Although the plants were selected in the Kansas environment, difference in seed yield between selected and unselected types was larger at the more moderate Minnesota location (Table 2). Total aboveground biomass was similar among genotypes, and higher than potential perennial biofuel crops for this region (Parrish and Fike, 2005). In Minnesota, the largest-seed genotypes had seed yield more than 200 g/m^2 (Table 2). These yields were obtained from the best genotypes in small hand-harvested plots, but they indicate that there is potential to obtain substantial seed yields from this perennial grass.

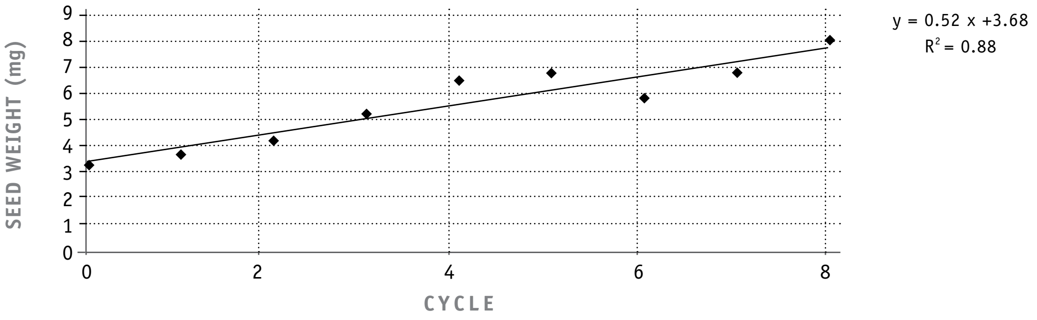
TABLE 2. PERFORMANCE OF INTERMEDIATE WHEATGRASS AT TWO LOCATIONS IN THE SECOND YEAR

Sets of clones (seed weight and seed yield) were compared with the starting population (Cycle 0) and a forage cultivar (Clarke). Predicted means from a mixed model, ± standard errors, are presented.

POPULATION	BIOMASS				SEED YIELD				SEED WEIGHT			
	MINNESOTA		KANSAS		MINNESOTA		KANSAS		MINNESOTA		KANSAS	
	g m^{-2}								mg $seed^{-1}$			
Cycle 0	1 690	±160	1 650	±160	84	±14	68	±14	5.73	±0.39	4.10	±0.39
Clarke	2 000	±160	2 170	±160	117	±14	49	±14	5.10	±0.39	3.73	±0.39
Seed Weight	2 380	±220	1 660	±110	212	±19	88	±10	9.26	±0.87	7.57	±0.66
Seed Yield	1 830	±140	1 810	±100	192	±12	100	±9	7.60	±0.68	6.07	±0.62

Since 2003 we have been performing mass selection for seed size. Initially, we began by harvesting a set of plants and obtaining the naked seed by floating off seed remaining in hulls. The naked seed was then passed through a set of sieves to obtain the largest seeds. These were planted, and then the process was repeated in the following year. After three cycles of selection, we began using a machine to automatically weigh each naked seed and sort out the largest seeds. We became concerned that this selection method might cause increased see sterility, since seed number and size are often inversely correlated (Sadras, 2007). So beginning with the fifth selection cycle we threshed each plant separately to obtain a seed yield per plant. Seed from the lowest-yield plants was discarded prior to sorting out the largest naked seed for selection. The selection intensity has varied from year to year, depending on seed yield. But in the later selection cycles about 120 plants have been established each cycle, selected out of about 40 000 naked seeds harvested. Seed size has fluctuated over the years depending on growing conditions and selection protocol. But the trend of increasing seed size has been strong (Figure 1). Eight cycles of selection have more than doubled seed weight when grown in a spaced plant selection nursery.

FIGURE 1. RESPONSE OF INTERMEDIATE WHEATGRASS TO MASS SELECTION BASED ON INDIVIDUAL SEED WEIGHT



Intermediate wheatgrass breeding in Manitoba

In the spring of 2011, a 4 500 plant nursery was established at the Carman, Manitoba research farm of the Department of Plant Science at the University of Manitoba. Accessions from the Germplasm Resources Information Network (GRIN) system of the United States Department of Agriculture (USDA) plus advanced generation materials from the Land Institute were planted.

The winter of 2011 to 2012 was relatively short with snowfall arriving in early December 2011 and snowmelt completed by 13 March 2012 on our Carman, Manitoba plots. For six days beginning on 19 March 2012, the daytime high was at least 19.9°C on four days with low temperatures above 0°C for the six days. This was followed by a four day period beginning 9 April 2012 where low temperatures were -5.5°C or lower. Approximately 50 percent of the 4 500 plants in the field sustained severe die-back. An additional 15 percent lodged prior to flowering, mostly due to reduced crown density caused by low temperature damage. The winter of 2012 to2013 was by comparison long and cold. Overnight lows below 0°C began on 31 October 2012 and lasted until 25 April 2013. Snow cover remained until 6 May 2013. An additional 15 percent of the remaining plants were lost due to these winter conditions. We have now experienced two sets of vastly divergent winter conditions and we now feel that we have excellent materials from which to base our selections. Yield stability will be evaluated utilizing the yields from the first two harvests (2012 and 2013). In 2013, some plants were removed from the nursery due to visually high levels of ergot.

Selection has taken place on the materials received from The Land Institute. A subsample of 100 plants was chosen at random to encompass the range of plant morphology within the nursery to investigate yield and its components. A comparison of the two sources indicates that the increased grain production has resulted in a higher harvest index (Figure 2). This has in part been achieved by a reduction for reduced spread (Figure 3). A major result of selection appears to be the increase in the seed yield per unit area of the plant (Figure 4). Initial selection will be based upon consistency of yield between years with an emphasis towards high yield per unit area. This would appear to lead towards a reduced plant size (at least plant area) and potentially higher yields in solid-seeded plantings.

Plant establishment greatly impacts competitiveness. The ability to rapidly emerge from the soil and compete with other species will impact productivity. Six half-sibling families were selected to study the effect of seed size on germination and emergence.

FIGURE 2. RELATIONSHIP BETWEEN HARVEST INDEX AND SEED YIELD PLANT^{-1} IN IMPROVED AND NON-IMPROVED INTERMEDIATE WHEATGRASS

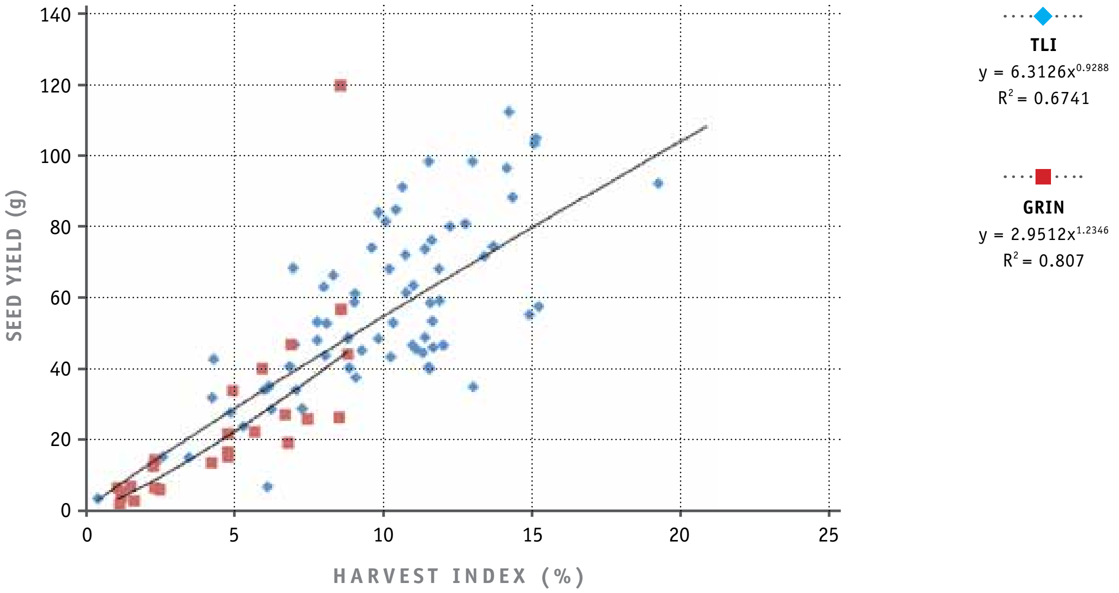


FIGURE 3. RELATIONSHIP BETWEEN PLANT AREA AND SEED YIELD PLANT^{-1} IN IMPROVED AND NON-IMPROVED INTERMEDIATE WHEATGRASS

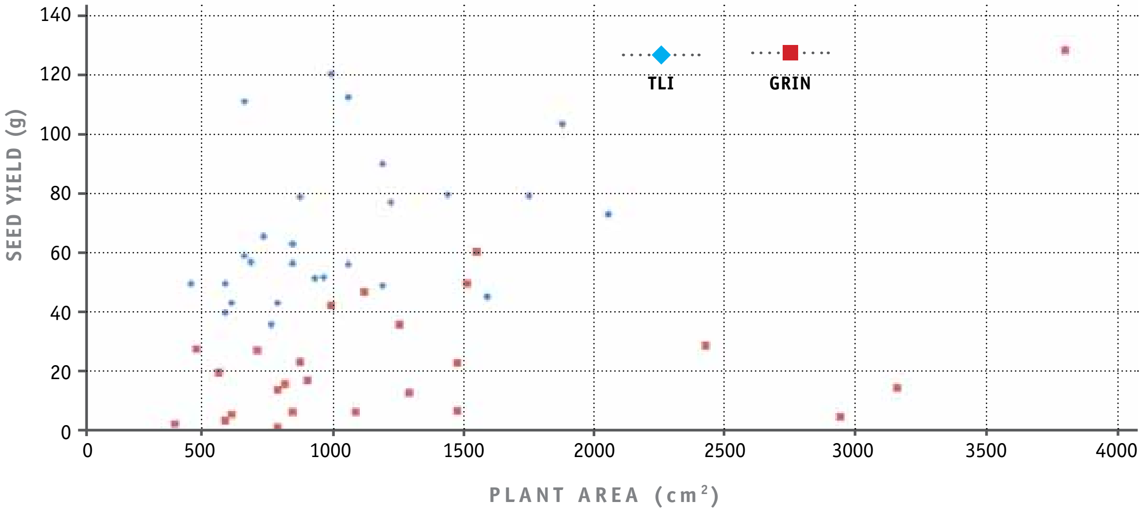


FIGURE 4. RELATIONSHIP BETWEEN PLANT AREA AND SEED YIELD CM^{-2} OF PLANTS IN IMPROVED AND NON-IMPROVED INTERMEDIATE WHEATGRASS

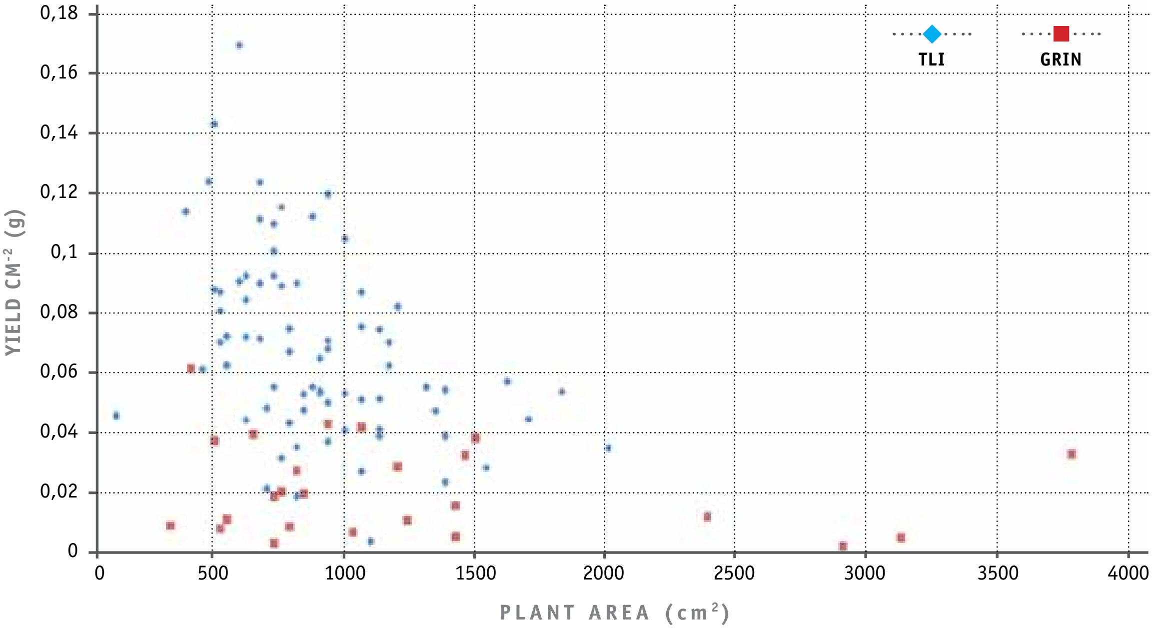
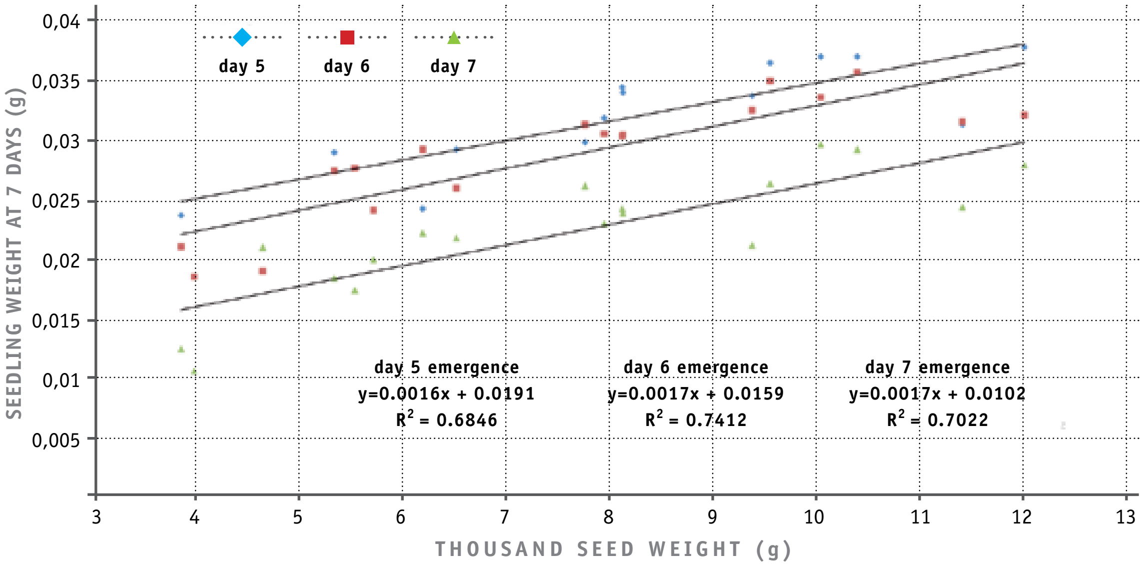


FIGURE 5. RELATIONSHIP BETWEEN THOUSAND SEED WEIGHT AND INITIAL BIOMASS ACCUMULATION IN INTERMEDIATE WHEATGRASS IN THE SEVEN DAYS AFTER EMERGENCE



Germination tests were conducted to ascertain seed viability, and emergence tests from a 2 cm depth (both field and greenhouse) were used to study relative ability to establish. Correlation between the germination tests and greenhouse emergence was high (r = 0.901), while emergence in the greenhouse and field was r = 0.781. Field emergence and germination tests showed the lowest correlation (r = 0.623). Emergence was not necessarily related to seed weight and the ability of plants to amass dry weight was relatively uniform for the first three days of emergence (Figure 5). The earlier the emergence, the greater the seedling weight after seven days of growth (Figure 5). This may be attributed to less energy being expended during emergence, thus resulting in a higher intercept value. Alternatively, early-emerging seedlings may possess genes contributing to rapid growth both before and after emergence.

Improving intermediate wheatgrass quality

Intermediate wheatgrass grain is currently inferior to wheat for most potential uses. Many of the limitations are due to a small grain size and the resulting high fibre and protein of whole grain flour. Small seed produces a low milling yield of white flour. Small grain size is being addressed through breeding, but an additional limitation is the low gluten quality that limits utility in raised breads. We have found that the low gluten quality of intermediate wheatgrass may be due to a very low quantity of HMW-GS.

In fact, HMW-GS genes have been transferred from intermediate wheatgrass to wheat to improve quality (Li *et al.* 2013; Niu *et al.* 2011). Therefore, we investigated the diversity and potential function of HMW-GS alleles in intermediate wheatgrass. Using gene cloning, protein separation and identification, and sequence alignments, we were able to identify five HMW-GS genes and their allelic variants in intermediate wheatgrass plants. Because the species is diverse and heterozygous, one or two allelic variants of each gene were detected in each plant, and an average of five HMW-GS proteins were found in individual plants.

Protein chemistry has demonstrated that all the identified HMW-GS were involved in protein polymers. Sequence alignments showed that two genes share high identities (>96 percent) with the HMW-GS genes from the D-genome of common wheat. We predict that with selection for high expression of superior allelic variants, HMW-GS might contribute to the bread-making quality of intermediate wheatgrass.

Intermediate wheatgrass molecular work

EST markers were developed for allohexaploid intermediate wheatgrass (*Thinopyrum intermedium*) and three closely related diploid species *Pseudoroegneria spicata, Thinopyrum bessarabicum*, and *Thinopyrum elongatum* (Table 3).

TABLE 3. DESCRIPTION OF ESTS AND EST MARKER RESOURCES FOR INTERMEDIATE WHEATGRASS

	P. spicata	*Th. intermedium*	*Th. elongatum*	*Th. bessarabicum*
Platform	Sanger	Roche 454	Roche 454	Roche 454
Reads (avg. bp)	16 128	566 681 (432)	447 936 (421)	364 193 (426)
Contigs (avg. bp)	6 450 (1 017)	29 851 (637)	30 858 (650)	15 700 (701)
Singletons (avg. bp)	2 330 (670)	45 760 (374)	38 216 (365)	22 709 (348)
EST isotigs (avg. bp)	8 780 (924)	75 611 (478)	69 074 (492)	38 409 (492)
Total bp	8 120 750	36 153 671	34 024 034	18 928 290
EST-SSR markers	1 375	672	596	384
Hv1[a]	152	98	70	68
Hv2	185	109	87	45
Hv3	172	106	90	54
Hv4	176	100	87	55
Hv5	204	98	95	77
Hv6	158	70	76	41
Hv7	187	91	71	44

a Correspondence of EST-SSR markers to the seven barley chromosomes (Hv1 to Hv7).

It is thought that the three subgenomes of allohexaploid intermediate wheatgrass may include one *Pseudoroegneria* (**St**) genome and two *Thinopyrum* (**E**) genomes similar to *Th. elongatum* (**E^{e}**) and/or *Th. bessarabicum* (**E^{b}**) genomes (Liu and Wang, 1993; Zhang *et al.* 1996; Zhang *et al.* 1997). A total of 1 375 Pspi EST-SSR primer pairs were previously developed from *P. spicata* (Table 3) (Bushman *et al.* 2008). Another 1 652 EST-SSR markers were recently designed from Roche 454 ESTs from intermediate wheatgrass, *Th. elongatum*, and *Th. bessarabicum* (Table 3). All of the *Thinopyrum* EST-SSR markers were designed from ESTs corresponding to one of the seven barley (Hv) chromosomes (Table 3). Likewise, most of the previously designed *P. spicata* EST-SSR markers also correspond to one of the seven barley (Hv) chromosomes (Table 3) based on alignments to the barley genome sequence (The International Barley Genome Sequencing Consortium, 2012).

The *P. spicata* and intermediate wheatgrass EST-SSR primers have been tested for amplification and/or polymorphism in intermediate wheatgrass and related species. Approximately 79 percent (1 083) of the *P. spicata* EST-SSR primers amplified products from *P. spicata* (Bushman *et al.* 2008). More recent experiments also showed that about 80 percent (1086) of the *P. spicata* EST-SSR primers amplified products from both *P. spicata* and intermediate wheatgrass. Similarly, about 84 percent (564) of the intermediate wheatgrass EST-SSR primers amplified products from intermediate wheatgrass. Moreover, about 68 percent of the intermediate wheatgrass EST-SSR primers amplified products from Chinese Spring wheat.

A combination of EST-SSR and AFLP markers will be used to genotype an experimental mapping population comprises 268 full-sib progeny derived from reciprocal crosses of two experimental genotypes, M35 × M26 (137) or M26 × M35 (131). The M26 genotype derives from a cross of C3-3471 × C3-3941. The C3-3941 genotype was also the female parent of M35. So far, six AFLP primers have also been genotyped on M26, M35, C3-3471, C3-3941 and 268 full-sib mapping progeny. These six AFLP primers detected 133 AFLP markers that were polymorphic between M35 and M26 with an average marker frequency of 0.52 among the 268 progeny. These six AFLP primers also detected 75 AFLP markers that were present in both M35 and M26 with an average marker frequency of 0.76 among the 268 progeny. Thus, a total of 208 polymorphic markers showing expected 1:1 or 3:1 segregation ratios were detected using the first six AFLP primer pairs. Another 13 AFLP markers that were present in both M35 and M26 showed marker frequencies greater than 0.9, which could be the result of segregation distortion or non-disomic inheritance. However, initial results were largely consistent with disomic inheritance. Although the mapping population has not yet been genotyped with EST-SSR markers, the M26, M35, C3-3471, and C3-3941 parents and grandparents have been screened using the 672 intermediate wheatgrass EST-SSR markers. Nearly 17 percent of the 564 amplified markers showed clear polymorphism between the M26 and M35 parents of this intermediate wheatgrass mapping population. Thus, we estimate that approximately 440 EST-SSR markers will be informative if this rate of polymorphism applies to *P. spicata*, *Th. elongatum*, and *Th. elongatum* EST-SSR markers.

Genotyping-by-sequencing (GBS) combines molecular marker discovery and genotyping and has been successfully used in species with large, complex genomes, such as barley and wheat. Intermediate wheatgrass is an allohexaploid with an estimated 14 GB genome and no reference genome. The combination of a large and understudied genome makes genetic studies and genomic assisted breeding for intermediate wheatgrass challenging. To address these constraints, we are utilizing the GBS approach to discover SNPs, and then using them to construct a genetic map and map important agronomic traits.

GBS involves simultaneously sequencing DNA from many individuals, but only specific sites in the organism's genome. The GBS approach increases the sequence coverage per site and dramatically reduces the sequencing cost per individual. These specific sites are targeted by restriction enzymes; for intermediate wheatgrass, we are using the restriction enzyme *PstI*, which identifies all sites in the genome with the DNA sequence 'CTGCAG' and cuts the DNA between the A and G nucleotides. A DNA barcode (unique 5-10 bp sequence) and barcode adapter are attached to the DNA fragments from each individual. DNA from many individuals is then pooled and sequenced as if it were from one individual (we sequence 96 individuals at a time). DNA is currently sequenced using Illumina's HiSeq platform and SNPs are called using the Universal Network Enabled Analysis Kit (UNEAK) bioinformatics pipeline.

The genetic map analysis began with ~3000 high quality SNPs from 285 selfs, part of a larger population of ~750 selfs from one individual. Intermediate wheatgrass is primarily outcrossing, but a large effort was concentrated on selfing an individual with an important set of agronomic traits: free-threshing, large seed and non-shattering. Preliminary phenotypic analyses suggest that these traits are correlated, dominant, and segregating in the selfed population. The framework map has been constructed with MapMaker and includes 859 SNPs from 271 individuals, divided into 22 linkage groups (there are 21 chromosomes). One small linkage group shows linkage with two larger linkage groups, but cannot be precisely placed. Preliminary analyses suggest that GBS can be utilized in intermediate wheatgrass for *de novo* genotyping to develop high-density genetic maps and genomic selection models for crop improvement.

Genetic studies of intermediate wheatgrass are complicated by the plant's high degree of heterozygosity, hexaploid nature, and self-incompatibility. Future work would be simplified by access to completely inbred individuals. We have been attempting to obtain fully homozygous individuals by producing doubled haploids through another culture. So far approximately 20 000 anthers have been cultured. Hundreds of calluses have formed. Dozens of calluses have initiated shoots, but most have been albino. We have obtained 5 plantlets that are green. The haploid nature of three young plants has been confirmed, and we are attempting to double their chromosome numbers with colchicine.

CONCLUSIONS

"Perennial wheat" hybrids and intermediate wheatgrass are currently at far ends of a spectrum. While the hybrid types have good grain yield, large seed, and other favourable traits, they remain functionally annual in most environments. On the other hand, intermediate wheatgrass is a vigorous perennial that is easily grown in diverse environments. But it lacks adequate seed yield, seed size, and grain quality to be commercially successful. Working from both directions, we expect that these programs will meet somewhere in the middle. Along the way, much of what is learned and developed in one approach will be useful to the other. For instance, genetic maps developed for intermediate wheatgrass will be critical for perennial wheat development. Understanding the physiology, morphology, phenology, and genomic functioning of perennial wheatgrass will be useful to those developing perennial wheat. Similarly, much that is known about the quality, pathology, yield, and genomics of wheat is being readily applied to intermediate wheatgrass.

We should not choose between the strategies of wide hybridization or domestication in the development of perennial small grains. The most rapid progress will be made by simultaneously working from both ends of the spectrum in order to produce perennial grain crops. Whether the perennial grain of the future will be wheat with grass-like traits added or a perennial grass with the addition of wheat-like traits is a question that we need not dwell on. What is clear is that progress is being made at an accelerating pace toward the day when useful, high-yield, long-lived small grains are a reality.

REFERENCES

Bushman, B.S., Larson, S.R., Mott, I.W., Cliften, P.F., Wang, R.R.C., Chatterton, N.J., Hernandez, A.G., Ali, S., Kim, R.W., Thimmapuram, J., Gong, G., Liu, L. & Mikel, M.A. 2008. Development and annotation of perennial *Triticeae* ESTs and SSR markers. *Genome.* 51**:** 779-788.

Chen, Q. 2005. Detection of alien chromatin introgression from *Thinopyrum* into wheat using S genomic DNA as a probe – A landmark approach for *Thinopyrum* genome research. *Cytogenet Genome Res.* 109: 350–359

Cox, T.S., Bender, M.H., Picone, C., Van Tassel, D.L., Holland, J.H., Brummer, E.C., Zoeller, B.E., Paterson, A.H. & Jackson, W. 2002. Breeding perennial grain crops. *Critical Reviews in Plant Sciences.* 21: 59–91.

Cox, T.S., Glover, J.D., Van Tassel, D.L., Cox, C.M. & DeHaan, L.R. 2006. Prospects for developing perennial grain crops. *Bioscience.* 56: 649–659.

Cox, T. S., Van Tassel, D. L., Cox, C.M. & DeHaan, L.R. 2010. Progress in breeding perennial grains. *Crop and Pasture Science.* 6: 513-521.

Ellneskog-Staam, P. & Merker, A. 2002. Chromosome composition, stability and fertility of alloploids between *Triticum turgidum* var. *carthlicum* and *Thinopyrum junceiforme*. *Hereditas.* 136: 59-65.

Gill, B.S. 1991. Nucleocytoplasmic interaction (NCI) hypothesis of genome evolution and speciation in polyploid plants. In: Sasakurma, T. (ed.) *Proceedings of the Kihara Memorial International Symposium on Cytoplasmic Engineering in Wheat.* Yokohama, Japan, 48–53.

Hayes, R.C., Newell, M.T., DeHaan, L.R., Murphy, K.M., Crane, S., Norton, M.R., Wade, L.J., Newberry, M., Fahim, M., Jones, S.S., Cox, T.S. & Larkin, P.J. 2012. Perennial cereal crops: An initial assessment of wheat derivatives. *Field Crops Research.* 133: 68–89.

Jackson, W. 1980. New roots for agriculture. University of Nebraska Press.

Jaikumar, N.S., Snapp, S.S., Murphy, K. & Jones, S.S. 2012. Agronomic assessment of perennial wheat and perennial rye as cereal crops. *Agronomy Journal.* 104: 1716–1726.

Jones, T.A., Zhang, X. & Wang, R.R.C. 1999. Genome characterization of MT-2 perennial and OK-906 annual wheat x intermediate wheatgrass hybrids. *Crop Science.* 39: 1041-1043.

Li, G.R., Liu, C., Li, C.H., Zhao, J.M., Zhou, L., Dai, G., Yang, E.N. & Yang, Z.J. 2013 Introgression of a novel *Thinopyrum intermedium* St-chromosome-specific HMW-GS gene into wheat. *Molecular Breeding.* 31: 843-853.

Liu, Z.W. & Wang, R.R.C. 1993. Genome analysis of *Elytrigia caespitosa*, *Lophopyrum nodosum*, *Pseudoroegneria geniculata* ssp. scythica, and *Thinopyrum intermedium* (*Triticeae: Gramineae*). *Genome.* 36: 102-111.

Ma, X. & Gustafson, J.P. 2008. Allopolyploidization-accommodated genomic sequence changes in triticale. *Annals Botany.* 101: 825–832.

Murphy, K.M., Lyon, S.R., Balow, K.A. & Jones, S.S. 2010. Post-sexual cycle regrowth and grain yield in *Thinopyrum elongatum* x *Triticum aestivum* amphiploids. *Plant Breeding.* 129: 480–483.

Niu, Z.X., Klindworth, D.L., Wang, R.R.C., Jauhar, P.P., Larkin, P.J. & Xu, S.S. 2011. Characterization of HMW glutenin subunits in *Thinopyrum intermedium*, *Th. bessarabicum*, *Lophopyrum elongatum*, *Aegilops markgrafii*, and their addition lines in wheat. *Crop Science.* 51: 667-677.

Ozkan, H. & Feldman, M. 2009. Rapid cytological diploidization in newly formed allopolyploids of the wheat (*Aegilops-Triticum*) group. *Genome.* 52: 926-934.

Parrish, D.J. & Fike, J.H. 2005. The biology and agronomy of switchgrass for biofuels. *Critical Reviews in Plant Sciences.* 24(5-6): 423-459.

Sadras. V.O. 2007. Evolutionary aspects of the trade-off between seed size and number in crops. *Field Crops Research*. 100: 125–138.

The International Barley Genome Sequencing Consortium. 2012. A physical, genetical and functional sequence assembly of the barley genome. *Nature*. 491: 711-716.

Tsitsin, N.V. 1978. Perennial Wheat. Trans. Li T *et al.* Beijing: Agriculture Press.

Turner, M.K., DeHaan, L.R., Jin, Y. & Anderson, J. A. 2013. Wheatgrass–wheat partial amphiploids as a novel source of stem rust and Fusarium head blight resistance. *Crop Science*. 53: 1994-2005.

Wagoner, P. 1990. Perennial grain new use for intermediate wheatgrass. *Journal of Soil and Water Conservation*. 45: 81-82.

Zhang, X.Y., Koul, A., Petroski, R., Ouellet, T., Fedak, G., Dong, Y.S. & Wang, R.R.C. 1996. Molecular verification and characterization of BYDV-resistant germplasm derived from hybrids of wheat with *Thinopyrum ponticum* and *Th. intermedium*. *Theoretical and Applied Genetics*. 93: 1033-1039.

Zhang, X.Y., Wang, R.R.C., Fedak, G. & Dong, Y.S. 1997. Determination of genome and chromosome composition of *Thinopyrum intermedium* and partial amphiploids of *Triticum aestivum X Th. intermedium* by GISH and genome specific RAPD markers. *Chinese Agricultural Science*. 1997: 71-80.

07

VIEWPOINT:
MULTIPLE-HARVEST SORGHUMS TOWARD IMPROVED FOOD SECURITY

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ABSTRACT

Perennial crops offer an avenue by which marginal lands might be brought into sustainable production while arresting or even reversing losses of ecological capital. The natural adaptation of sorghum to its native Sahelian region makes it essential to some of the poorest and most food-insecure human populations, dependent upon some of the world's most degraded soils. Increased demand for limited fresh water, together with rising global temperature and aridity, suggest that the inherent drought tolerance of sorghum will be of growing importance. With some genotypes already known to be suitable for ratoon crop production, and with two sources of perenniality, the *Sorghum* genus has become a model for dissecting the molecular control of ratooning and perenniality. We are pursuing two broad approaches to investigate the efficacy of ratoon/perennial sorghum production and to develop genotypes suitable for these production systems. First, the identification of diploid progeny from crosses between *S. bicolor* and *S. propinquum* offers a starting point for investigation of these traits in conventional sorghums, by crossing to elite sorghums and evaluation of resulting progeny (potentially also employing DNA-marker aided selection to increase the frequency of genotypes that contain known Quantitative trait loci (QTLs) for ratooning and/or perenniality-related traits). Second, crosses between tetraploid forms of *S. bicolor* and their naturally-tetraploid relative *S. halepense*, may offer the possibility to benefit from many traits that differentiate this widespread weedy/invasive plant from its progenitors. While initial efforts have focused on grain production, substantial forage/biomass is also produced by ratoon/perennial sorghums and in principle there appears to be no insurmountable obstacle to also developing perennial 'sweet sorghums' suitable for sugar production. Perennial cropping systems may be especially attractive to smallholder agriculture (particularly benefitting women farmers), in which poor seedling emergence and stand establishment can lead to crop failure and costs of seed, energy, and equipment are limiting factors. However, the benefits of perennials may also apply to intensive agriculture, especially in situations where erosion, nutrient leaching, and declining organic matter levels occur. A fringe benefit of increased knowledge of perenniality may be new tools and approaches by which to control widespread agricultural weeds. Comparative data from sorghum and rice suggest that findings with regard to perenniality may extrapolate broadly to a wide range of taxa, accelerating progress in implementing new production systems (or weed control systems) in other genera.

Keywords: ratoon crop, perennial, *Sorghum propinquum, Sorghum halepense*

INTRODUCTION TO SORGHUM

About 20 million years ago , a population of 20-chromosome grassy plants had a singularly bright future. An early branch diverged from this population ~10-15 million years ago, experiencing genome-wide duplication and genome size expansion accompanied by chromosome number reduction leading to the modern maize genome (Gaut, Clark, *et al.* 1997; Swigonova, Lai, *et al.* 2004). About 8-9 million years ago (Jannoo *et al.* 2007; Sobral *et al.*

1994) another branch diverged, also experiencing genome-wide duplication and leading to the modern genomes of Saccharum. It was perhaps the most important biomass/biofuels crop worldwide, and its close relative *Miscanthus*, which is among the highest-yielding herbaceous temperate biomass crops known (Heaton, Dohleman, et al. 2008). A third branch spawned lineages with a wide range of fates (Spangler *et al.* 1999). One being domesticated into cultivated sorghum, and another experiencing polyploidy to form one of the world's most aggressive weeds (*Sorghum halepense*).

Among these important grasses, all members of the Andropogoneae clade, the genome of *Sorghum bicolor* L. Moench (sorghum) is of central importance both as a botanical model and as a major crop. Sorghum is the most drought-resistant of the world's top five cereal crops, and an important dual-purpose (grain + straw) crop. Its drought resistance is essential in the United States Southern Plains that often receive too little rain for other grains. And in arid countries of northeast and West Africa, sorghum contributes as much as 26-39 percent of calories in the human diet (www.fao.org). Nonetheless, sorghum improvement lags that of maize, wheat and rice, each of which have more than doubled in worldwide average yield in the last 38 years. Sorghum yields only gained 51 percent (www.fao.org). Increased demand for limited fresh water, together with rising global temperature and aridity, suggest that sorghum will be of growing importance.

While grain is of special importance, the value of other sorghum products should not be underestimated. Sweet sorghum for stock feed (indirectly sustaining protein production) is a large and growing market, and the possibility of production systems based on perenniality combined with low/no-till farming are very attractive. Further, bioenergy sorghums are a promising source of cellulosic ethanol (Rooney *et al.* 2007). They are even better suited to tropical regions (such as much of Africa) than temperate regions, because a more continuous supply of feedstock is possible in the tropics.

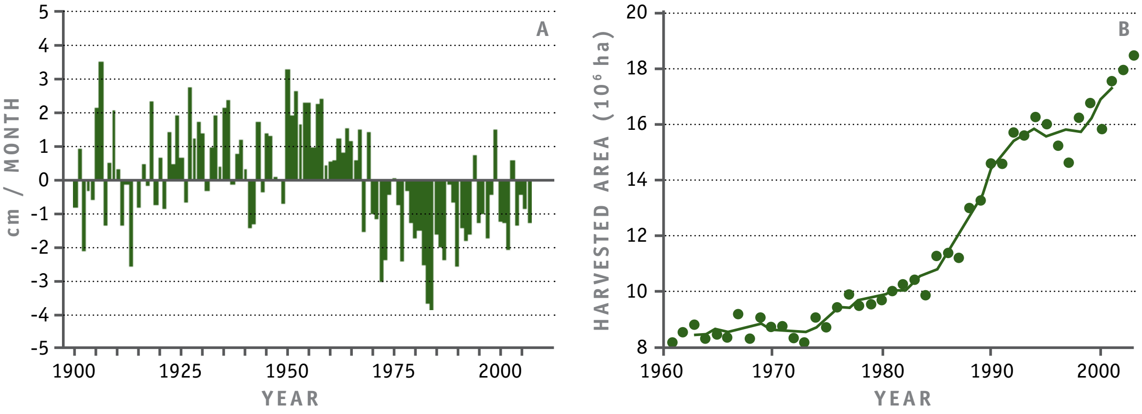
The sequenced ~740 Mb sorghum genome (Paterson *et al.* 2009) is a logical complement to that of *Oryza* (rice) for grass functional genomics. It is a representative of tropical grasses in which biochemical and morphological specializations ('C4' photosynthesis) improve carbon assimilation at high temperatures. The sorghum karyotype is suggested by parsimony to have been shared by the Andropogoneae common ancestor, with only ~3 percent differential sorghum-rice gene loss and less sorghum-rice structural rearrangement in 50 million years than sorghum-maize rearrangement in about 15 million years (Bowers *et al.* 2003). With 70 million years of 'abstinence' from genome duplication, functions of *S. bicolor* genes also may still resemble those of the common cereal ancestor.

Sorghum evolution and improvement have yielded a remarkable diversity of morphologies. Differences in inflorescence and seed morphology are used to classify *S. bicolor* races. Also they have been embellished by divergent selection applied to forms cultivated for purposes ranging from 'broomcorn' to the compact inflorescences and large seed of grain types. Divergent selection has also affected plant stature, with stalks of up to 6 metres being a key building material in Africa, but less than 1 metre favoured for mechanized harvest. Selection for carbohydrate rich seed/grain has increased harvest index (carbon partition to grain) in grain types, while selection for sugar-rich stalks and/or total biomass has reduced seed size and output in others to an extreme exemplified by *S. propinquum* (Figure 1). Human selection has been superimposed on rich natural variation in plant architecture and carbon allocation, for example ranging from one to 100+ tillers per crown, and branching along a tiller ranging from none to secondary, tertiary, and even quaternary. Its morphological and biochemical diversity makes sorghum the only crop that is compatible with fuel production from each of three routes, including starch (seed), cellulose (stalks), and sugar ('sweet' sorghums have sugar yields comparable to those of sugarcane).

FIGURE 1. COMPARISON OF ANNUAL *SORGHUM BICOLOR* AND PERENNIAL *S. PROPINQUUM*

(A) Crowns – including extensive rhizomes of *S. propinquum*.
(B) Seeds – those of *S. bicolor* grain types are large and carbohydrate rich, while those of *S. propinquum* are very small.

FIGURE 2. (A) SAHEL PRECIPITATION, 1900-2007; (B) SAHEL CROPPING AREA, 1960-2010



A: modified from Haywood *et al.*, 2013
B: *source*: Kandji *et al.*, 2006

Rich resources are in place to link sorghum phenotypic diversity to its molecular basis. More than 30 sorghum genomes and 40 transcriptomes, sampling all botanical races of cultivated sorghum, wild *S. bicolor*, and appropriate outgroups, have now been sequenced by the United States Department of Energy Joint Genome Institute. GBS has been performed on several reference collections (Morris *et al.* 2013), including two developed by the International Crops Research Institute for the Semi-Arid-Tropics (ICRISAT) that have been focal points of phenotyping for morphological variation, yield components, and drought responses (Upadhyaya *et al.* 2009).

OVERVIEW OF RESEARCH ON PERENNIALITY IN SORGHUM

The natural adaptation of sorghum to its native Sahelian region makes it essential to some of the poorest and most food-insecure human populations and dependent upon some of the world's most degraded soils. Years of above average rainfall were followed by drought in the Sahel starting in the late 1960s (Figure 2A). A dramatic increase in the Sahelian area devoted to crops began at that time, with falling yields necessitating greater effort to cultivate ever-more fragile lands to feed growing populations (Figure 2B). These considerations are thought to contribute greatly to the fact that the Sahel now includes some of the most degraded lands on the planet.

Recently, the potential benefits of perennial grain crops have gained wider recognition (Glover *et al.* 2010). Perennial crops are essential to bringing marginal lands into sustainable production (Cox *et al.* 2002; Scheinost, 2001; Wagoner, 1990), maximizing ecosystem productivity (Field, 2001) and minimizing losses of topsoil (Pimentel *et al.* 1995), water, and nutrients. Perennial cover is >50 times more effective than annual crops in maintaining topsoil (Gantzer *et al.* 1990), and 30-50 times more effective than annuals at preventing nitrogen losses (Randall and Mulla, 2001). Perennial cropping systems (Cox *et al.* 2006; Jackson and Jackson, 1999; Piper, 1998) may be especially attractive to smallholder agriculture, in which poor seedling emergence and stand establishment can lead to crop failure, and costs of seed, energy, and equipment are limiting factors. It also may benefit mechanized agriculture in situations where erosion, nitrate (and other nutrient) leaching, and declining organic matter levels occur.

The *Sorghum* genus has become a model for dissecting the molecular control of ratooning and perenniality largely due to the availability of the diploid perennial *S. propinquum* which is abundantly tillering and has rhizomes that contribute to perenniality (Hu *et al.* 2003; Jang *et al.* 2006; Jang *et al.* 2008; Paterson *et al.* 1995). Tillers and rhizomes both develop from axillary buds at the lowermost nodes of the erect leafy shoot of the plant, with acropetally increasing tendency to develop into tillers (Gizmawy *et al.* 1985). There is appreciable correspondence in the locations of QTLs determining tillering and rhizomatousness (Paterson *et al.* 1995), thought to be related to the number of buds available. The commitment of a bud to rhizome development, tiller development, or quiescence is genetically-determined (Paterson *et al.* 1995), and is associated with marked differences in gene expression (Jang *et al.* 2006). Finally, a high level of correspondence of rhizome QTLs between sorghum and rice (Hu *et al.* 2003), thought to have diverged from common ancestry about 50 million years ago, suggests that key genes conferring perenniality-related traits may be shared by many Poaceae cereals. In other words, fundamental information about these traits in one cereal may extrapolate broadly to many other cereals.

While no members of the cultivated species, *Sorghum bicolor,* are rhizomatous, there is variation among cultivars for ratoon crop productivity (Duncan *et al.* 1980; Duncan and Moss, 1987), and close relatives sugarcane and *Miscanthus* are usually grown as ratoon crops. Ratooning (regrowth) is a complex trait, showing quantitative inheritance (Duncan *et al.* 1980). Likewise, QTL mapping in an F2 population of a cross between an elite grain sorghum inbred BTx623 (later used for the reference sequence) and *S. propinquum*, showed regrowth to be closely related to both rhizomatousness and tillering, but was only able to account for 14-30 percent of phenotypic variance in these traits (Paterson *et al.* 1995). A recombinant inbred line population for this cross (Kong *et al.* 2013), now provides for replicated genetic analysis of productivity, and includes genotypes with a range of 'degrees' of ratoon cropping from near-zero to fully perennial. The recombinant inbred line population has been genetically mapped with both the population itself and map publicly available (Kong *et al.* 2013), and with three years of phenotyping for tillering, rhizomatousness, and basic metrics related to grain production (yield components) presently being analysed for peer-reviewed publication.

A relative of sorghum, *S. halepense*, is a wild perennial polyploid resulting from natural hybridization between *S. bicolor* (2n = 20) and *S. propinquum* (2n = 20), a wild perennial diploid native to Southeast Asia and estimated to have diverged from *S. bicolor* ~1-2 million years ago. *S. halepense* finds occasional use as forage and even food (seed/flour), but is most noted as one of the world's most noxious weeds, having spread from its west Asian centre of diversity across much of Asia, Africa, Europe, North and South America, and Australia. Its establishment in the United States of America is probably typical of its spread to other continents, including intentional introduction as a prospective forage and/or unintentional introduction as a contaminant of sorghum seedlots (McWhorter, 1971). However, while *S. bicolor* has remained largely confined to cultivation, *S. halepense* has readily naturalized – revealing genetic potential for adaptation that goes well beyond that of sorghum. *S. halepense* crosses readily with *S. bicolor* (Arriola and Ellstrand, 1996) –more than 90 percent of plants in naturalized *S. halepense* populations growing adjacent to long-term sorghum production fields contain alleles introgressed from sorghum (Morrell *et al.* 2005).

RESEARCH AGENDA

We are pursuing two broad approaches to investigate the efficacy of ratoon/perennial sorghum production, and to develop genotypes suitable for these production systems.

First, the identification of diploid progeny from crosses between *S. bicolor* and *S. propinquum* (Kong *et al.* 2013) offers a starting point for investigation of these traits in conventional sorghums, by crossing to elite sorghums and evaluation of resulting progeny (potentially also employing DNA-marker aided selection to increase the frequency of genotypes that contain known QTLs for ratooning and/or perenniality-related traits).

Second, crosses between tetraploid forms of *S. bicolor* and their naturally-tetraploid relative *S. halepense*, may offer the possibility to benefit from many traits that differentiate this widespread plant from its progenitors. While *S. halepense* is generally under selection for weediness-related attributes that are undesirable in annual row-crop production, some selection pressures may favour *S. halepense* alleles that are of potential value in sorghum, for example conferring rapid vegetative development and early flowering. An attractive but unproven hypothesis, which the ability of *S. halepense* to naturalize in a much wider range of environments than sorghum is cultivated may have been accompanied by selection for alleles associated with valuable attributes such as cold tolerance (for example in the northern United States of America and Russia), drought tolerance (Africa, Arabia, United States of America in the southwest), disease resistance (many) or other traits. Moreover, a host of data now show that unpredictable, presumably epistatic, effects of allele introgressions from seemingly undesirable sources can confer valuable traits to crops (Bernacchi *et al.* 1998a; Bernacchi *et al.* 1998b; Chee *et al.* 2005; Eshed and Zamir, 1995; Fridman *et al.* 2004; Fulton *et al.* 1997; Gur and Zamir, 2004; Schauer *et al.* 2006; Tanksley *et al.* 1996; Xiao *et al.* 1996; Zamir, 2001).

At present, we are phenotyping and analysing F2 populations, their F3 progeny and the progeny of backcrosses to *S. bicolor* for perenniality, plant architecture, yield components and domestication traits. While we find some of these tetraploids to have fecundity comparable to diploid sorghums and are potentially directly usable, another attractive route for their utilization would be to extract diploids with selected properties of *S. halepense* either by backcrossing or by the development of haploids.

IMPLICATIONS

The benefits of perenniality as a cropping system are receiving renewed interest. Perennial plants have four advantages over annuals: a longer growing season; better access to water and nutrients; more conservative use of nutrients and better adaptation to marginal lands (Cox *et al.* 2006). The first three of those characteristics are expressed on all landscapes, from prime farmland to poor or sloping soils unsuitable for annual cropping. The expansion of agriculture to provide plant biomass for production of fuels or chemical feedstocks with little or no carbon release (Fargione *et al.* 2008) will require greater utilization of marginal land (Tilman *et al.* 2009), maximizing ecosystem productivity (Field, 2001) and minimizing losses of topsoil (Pimentel *et al.* 1995), water, and nutrients.

The benefits of perenniality apply to food, as well as biomass, crops. Annual food crops have dominated the agricultural landscape since the time of the earliest farmers. In the last few decades alone, one-third of the planet's arable land has been lost to soil erosion (Pimentel *et al.* 1995). No-till production of annual crops, designed to control soil loss, has so far required increased use of herbicides. Direct-seeded annual cropping systems produce as high or higher nitrate emissions as tillage systems (Mummey *et al.* 1998). 'Natural systems agriculture', based on developing a "domestic prairie" with herbaceous, perennial grain-producing crops grown in polyculture (Jackson and Jackson, 1999; Piper, 1998), has been proposed as a means to produce food on otherwise marginal lands while arresting or even reversing losses of ecological capital.

In smallholder production systems that are widespread in regions where sorghum is a staple in the human diet, conservation-agriculture approaches may particularly benefit women farmers, by reducing dependence on duties generally performed by men (ploughing) and mitigating the need for duties performed by women and children (uprooting grass, de-stumping) (Milder *et al.* 2011). Moreover, women frequently are given marginal fields that should be left fallow (Kevane, 2011), for example those which will be the greatest beneficiaries of multi-cropping approaches. Male farmers, with access to generally higher levels of land, labour, and cash, have a somewhat greater tendency to grow cash crops (Gilbert *et al.* 2005) – further indicating that early adopters of reduced-cost, reduced-labour multi-cropping sorghum production systems may tend to be female. A reduced requirement for seed selection in multi-cropping production systems (which propagate vegetatively), may also reduce male investment as seed selection is largely male-dominated (Freeman and Boateng, 2012).

Perenniality plays two contrasting roles in agriculture, being: (i) a valuable asset in establishment and persistence of dense, productive stands of plants suitable for multiple harvests, and (ii) an essential component of "weediness" of many of our most noxious weeds. Better understanding of the biology of *S. halepense* itself, one of the most noxious weeds affecting world agriculture (Holm *et al.* 1977), may also be of high value. *S. halepense* is a major contaminant in sorghum seed production, an alternate host and means of over-wintering for pests and pathogens of both monocot and dicot crops. It is also a highly-effective competitor for sunlight and other resources, causing reductions in economic yield of 45 percent or greater in crops as diverse as monocots such as sugarcane (Millhollen, 1970) and dicots such as soybean (McWhorter and Hartwig, 1972). The first United States federal appropriation for weed control research targeted Johnsongrass (House Bill #121, 1900), yet it remains largely unchecked today.

The *S. halepense* problem constrains improvement of sorghum through biotechnology. *S. halepense* is a particular problem in and around sorghum fields because no known herbicide can selectively eliminate it without damaging the sorghum. Transformation of sorghum was first accomplished in 1993 (Casas *et al.* 1993), and introduction of transgenes for resistance to insects, diseases, or herbicides could have a major impact on sorghum productivity. However, the risk of transgene escape constrains the ability of regulatory agencies to approve the release of sorghum cultivars with transgenes that could enhance agricultural productivity.

Better understanding of the biology of *S. halepense* and associated new functional genomics tools might aid greatly in identification of genes, regulatory elements, and biochemical functions that are important to its weediness, particularly its rhizomes, but dispensable to other plant processes (Jang *et al.* 2006). Identification of such genes would provide the foundation to search for plant growth regulators that specifically target rhizomes, perhaps providing for control of rhizomatous weeds even in closely related crops such as *S. halepense* in sorghum. Moreover, better understanding of rhizome development may also benefit development of plant genotypes optimal for biomass/biofeedstock production on marginal lands, as well as improvement of a host of forage and turf grasses.

Our demonstration that most QTLs responsible for rhizomatousness in *Sorghum* and *Oryza* respectively, map to corresponding locations (Hu *et al.* 2003), suggests that *information about rhizomatousness from a few models (that are also major crops) may extrapolate broadly to a wide range of taxa*. Successes in clarifying the genetics of perenniality in this genus, may accelerate progress in implementing new production systems in other genera, and also to controlling a wide range of related agricultural weeds.

REFERENCES

Arriola, P.E. & Ellstrand, N.C. 1996. Crop-to-weed gene flow in the genus Sorghum (Poaceae): Spontaneous interspecific hybridization between johnsongrass, *Sorghum halepense*, and crop sorghum, *S. bicolor*. *American Journal of Botany*. 83: 1153-1159.

Bernacchi, D., Beck-Bunn, T., Emmatty, D., Eshed, Y., Inai, S., Lopez, J. Petiard, V., Sayama, H., Uhlig, J., Zamir, D., & Tanksley, S. 1998. Advanced back-cross QTL analysis of tomato. II. Evaluation of near-isogenic lines carrying single-donor introgressions for desirable wild QTL-alleles derived from Lycopersicon hirsutum and L-pimpinellifolium (vol 97, pg 170, 1998). *Theoretical and Applied Genetics.* 97: 1191-1196.

Bernacchi, D., Beck-Bunn, T., Eshed, Y., Lopez, J., Petiard, V., Uhlig, J., Zamir, D. & Tanksley, S.D. 1998a. Advanced backcross QTL analysis in tomato. I: Identification of QTLs for traits of agronomic importance from Lycopersicon hirsutum. *Theoretical and Applied Genetics*. 97:381-397.

Bernacchi, D., Beck-Bunn, T., Emmatty, D., Eshed, Y., Inai, S., Lopez, J., Petiard, V., Sayama, H., Uhlig, J., Zamir, D. & Tanksley, S.D. 1998b. Advanced backcross QTL analysis in tomato. II: Evaluation of near-isogenic lines carrying single-donor introgressions for desirable wild QTL-alleles derived from Lycopersicon hirsutum and L. pimpinellifolium. *Theoretical and Applied Genetics.* 97: 170-180.

Bowers, J.E., Abbey, C., Anderson, S., Chang, C., Draye, X., Hoppe, A.H., Jessup, R., Lemke, C., Lennington, J., Li, Z.K., Lin, Y.R., Liu, S.C., Luo, L.J., Marler, B.S., Ming, R.G., Mitchell, S.E., Qiang, D., Reischmann, K., Schulze, S.R., Skinner, D.N., Wang, Y.W., Kresovich, S., Schertz, K.F. & Paterson, A.H. 2003. A high-density genetic recombination map of sequence-tagged sites for Sorghum, as a framework for comparative structural and evolutionary genomics of tropical grains and grasses. *Genetics* 165: 367-386.

Casas, A.M., Kononowicz, A.K., Zehr, U.B., Tomes, D.T., Axtell, J.D., Butler, L.G., Bressan, R.A. & Hasegawa, P.M. 1993.Transgenic sorghum plants via microprojectile bombardment. *Proceedings of the National Academy of Sciences of the United States of America.* 90(23):11212–11216.

Chee, P., Draye, X., Jiang, C.X., Decanini, L., Delmonte, T.A., Bredhauer, R., Smith, C.W. & Paterson, A.H. 2005. Molecular dissection of interspecific variation between Gossypium hirsutum and Gossypium barbadense (cotton) by a backcross-self approach: I. Fiber elongation. *Theoretical and Applied Genetics.* 111: 757–763.

Chee, P.W., Draye, X., Jiang, C.X., Decanini, L., Delmonte, T.A., Bredhauer, R., Smith, C.W. & Paterson, A.H. 2005. Molecular dissection of phenotypic variation between Gossypium hirsutum and Gossypium barbadense (cotton) by a backcross-self approach: III. Fiber length. *Theoretical and Applied Genetics111*: 772–781.

Cox, T.S., Bender, M., Picone, C., Van Tassel, D.L., Holland, J.B., Brummer, E.C., Zoeller, B.E., Paterson, A.H. & Jackson, W. 2002. Breeding perennial grain crops. *Critical Reviews in Plant Sciences*. 21: 59-91.

Cox, T.S., Glover, J.D., Van Tassel, D.L., Cox, C.M. & DeHaan, L.R. 2006. Prospects for developing perennial grain crops. *BioScience.* 56: 649-659.

Draye, X., Chee, P., Jiang, C.X., Decanini, L., Delmonte, T.A., Bredhauer, R., Smith, C.W. & Paterson, A.H. 2005. Molecular dissection of interspecific variation between Gossypium hirsutum and G. barbadense (cotton) by a backcross-self approach: II. Fiber fineness. *Theoretical and Applied Genetics.* 111: 764–771.

Duncan, R.R., Miller, F.R. & Bockholt, A.J. 1980. Inheritance of tiller regrowth in ratooned sorghum. *Canadian Journal of Plant Science*. 60: 473-478.

Duncan, R.R., & Moss, R.B. 1987. Comparative yields of ratoon cropped temperately and tropically adapted grain-sorghum hybrids. *Crop Science.* 27: 569-571.

Eshed, Y. & Zamir, D. 1995. An introgression line population of lycopersicon pennellii in the cultivated tomato enables the identification and fine mapping of yield-associated qtl. *Genetics.* 141: 1147-1162.

Fargione, J., Hill, J., Tilman, D., Polasky, D. & Hawthorne, P. 2008. Land clearing and the biofuel carbon debt. *Science.* 319: 1235-1238.

Field, C.B. 2001. Sharing the garden. *Science*. 294: 2490-2491.

Freeman, C.K. & Boateng, S.D. 2012. Gender and cultural dimensions of sorghum seed management in north-east Ghana. *Global Journal of Biology, Agriculture and Health Sciences.* 1: 4-9.

Fridman, E., Carrari, F., Liu, Y.S., Fernie, A.R. & Zamir, D. 2004. Zooming in on a quantitative trait for tomato yield using interspecific introgressions. *Science.* 305: 1786-1789.

Fulton, T.M., BeckBunn, T., Emmatty, D., Eshed, Y., Lopez, J., Petiard, V., Uhlig, J., Zamir, D. & Tanksley, S.D. 1997. QTL analysis of an advanced backcross of Lycopersicon peruvianum to the cultivated tomato and comparisons with QTLs found in other wild species. *Theoretical and Applied Genetics.* 95: 881-894.

Gantzer, C.J., Anderson, S.H., Thompson, A.L. & Brown, J.R. 1990. Estimating soil erosion after 100 years of cropping on Sanborn Field. *Journal of Soil and Water Conservation.* 45: 641-644.

Gaut, B.S., Clark, L.G., Wendel, J.F. & Muse, S.V. 1997. Comparisons of the molecular evolutionary process at rbcL and ndhF in the grass family (Poaceae). *Molecular Biology and Evolution. 14*: 769-777.

Gilbert, R., Sakala, W.D. & Benson, T.D. 2005. Gender analysis of a nationwide cropping system trial survey in Malawi. *African Studies Quarterly*. 6: web.africa.ufl.edu/asq/v6/v6i1a9.htm

Gizmawy, I., Kigel, J.,Koller, D. & Ofir, M. 1985. Initiation, orientation, and early development of primary rhizomes in *Sorghum halepense* (L.) *Annals of Botany*. 55: 343.

Glover, J.D., Reganold, J.P., Bell, L.W., Borevitz, J., Brummer, E.C., Buckler, E.S., Cox, C.M., Cox, T.S., Crews, T.E., Culman, S.W., DeHaan, L.R., Eriksson, D., Gill, B.S., Holland, J., Hu, F., Hulke, B.S., Ibrahim, A.M.H., Jackson, W., Jones, S.S., Murray, S.C., Paterson, A.H., Ploschuk, E., Sacks, E.J., Snapp, S., Tao, D., Van Tassel, D.L., Wade, L.J., Wyse, D.L. & Xu, Y. 2010. Increased food and ecosystem security via perennial grains. *Science.* 328: 1638-1639.

Gur, A. & Zamir, D. 2004. Unused natural variation can lift yield barriers in plant breeding. *Plos Biology*. 2: 1610-1615.

Hash, C.T., Ramu, P., Folkertsma, R.T., Upadhyaya, H.D., Billot, C., Rami, J-F., Deu, M., Gardes, L., Rivallan, R., Li, Y., Wang, T. & Lu, P. 2008. Diversity analysis of the sorghum global composite collection and reference set. *2008 Annual Research Meeting Generation Challege Programme.* Bangkok, Thailand.

Haywood, J.M., Jones, A., Bellouin, N. & Stephenson, D. 2013. Asymmetric forcing from stratospheric aerosols impacts Sahelian rainfall. *Nature Climate Change.* 3: 660-665. doi:10.1038/nclimate1857.

Heaton, E.A., Dohleman, F.G. Long, S.P. 2008. Meeting US biofuel goals with less land: the potential of Miscanthus. *Global Change Biology.* 14: 2000-2014.

Holm, L.G., Plucknett, D.L., Pancho, J.V. & Herberger, J.P. 1977. The world's worst weeds: distribution and biology.*University Press of Hawaii*. Honolulu, HI.

Hu, F.Y., Tao, D.Y. Sacks, E. Fu, B.Y. Xu, P., Li, J., Yang, Y., McNally, K., Khush, G.S., Paterson, A.H. & Li, Z-K. 2003. Convergent evolution of perenniality in rice and sorghum. *Proceedings of the National Academy of Sciences of the United States of America.* 100: 4050-4054.

Jackson, W. & Jackson, L.L. 1999. Developing high seed yielding perennial polycultures as a mimic of mid-grass prairie. In: Lefroy, E.C., Hobbs, R J., O'Connor, M. H., & Pate, J. S., editors, *Agriculture as a Mimic of Natural Systems*. Kluwer Academic Publishers, Dordrecht, Netherlands. pp. 1-37.

Jang, C.S., Kamps, T.L Skinner, D.N., Schulze, S.R., Vencill, W. & Paterson, A.H. 2006. Sorghum genes with rhizome-enriched expression: functional classification, genomic organizatin, putative cis-acting regulatory elements, and relationship to QTLs. *Plant Physiology*. 142: 1148-1159.

Jang, C.S., Kamps, T.L., Tang, H., Bowers, J.E., Lemke, C. & Paterson, A.H. 2008. Evolutionary fate of rhizome-specific genes in a non-rhizomatous Sorghum genotype. *Heredity.* 102: 266-273.

Jannoo, N., Grivet, L., Chantret, N., Garsmeur, O., Glaszmann, J.C., Arruda, P. & D'Hont, A. 2007. Orthologous comparison in a gene-rich region among grasses reveals stability in the sugarcane polyploid genome. *The Plant Journal*. 50: 574-585.

Kandji, S.T., Verchot, L. & Mackensen, J. 2006. *Climate Change and Variability in the Sahel Region: Impacts and Adaptation Strategies in the Agricultural Sector*. United Nations Environment Program (UNEP), and World Agroforestry Center (ICRAF), Nairobi, Kenya. p. 47.

Kevane, M. 2011. Gendered production and consumption in rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*. 109: 12350-12355. doi: 10.1073/pnas.1003162108.

Kong, W., Jin, H., Franks, C.D., Kim, C., Bandopadhyay, R., Rana, M.K., Auckland, S.A., Goff, V.H., Rainville, L.K., Burow, G.B., Woodfin, C., Burke, J.J. & Paterson, A.H. 2013. Genetic analysis of recombinant inbred lines for *Sorghum bicolor* x *S. propinquum*. *G3*. 3: 101-108.

McWhorter, C.G. 1971. Introduction and spread of Johnsongrass in the United States. *Weed Science*. 19: 496.

McWhorter, C.G. & Hartwig, E.E. 1972. Competition of johnsongrass and cocklebur with six soybean varieties. *Weed Science*. 20: 56-59.

Milder, J.C., Majanen, T. & Scherr, S.J. 2011. Performance and potential of conservation agriculture for climate change adaptation and mitigation in sub-Saharan Africa. An assessment of WWF and CARE projects in support of the WWF-CARE Alliance's Rural Futurres Initiative. (Available at https://foodgrainsbank.merchantsecure.com/uploads/CARE-WWF-EcoAgriculture_Conservation_Agriculture_in_Sub-Saharan_Africa_FINAL_REPORT_2011- 02-2811.pdf). p. 90.

Millhollen, R.W. 1970. MSMA for johnsongrass control in sugarcane. *Weed Science*. 18: 333.

Morrell, P.L., Williams-Coplin, D., Bowers, J.E., Chandler, J.M. & Paterson, A.H. 2005. Crop-to-weed introgression has impacted allelic composition of johnsongrass populations with and without recent exposure to cultivated sorghum. *Molecular Ecology*. 14: 2143-2154.

Morris, G.P., Ramu, P., Deshpande, S.P., Hash, C.T., Shah, T., Upadhyaya, H.D., Lizarazu, O., Brown, P.J., Acharya, C.B., Mitchell, S.E., Harriman, J., Glaubitz, J.C., Buckler, E.S. & Kresovich, S. 2013. Population genomic and genome-wide association studies of agroclimatic traits in sorghum. *Proceedings of the National Academy of Sciences of the United States of America*. 110: 453-458.

Mummey, D.L., Smith, J.L. & Bluhm, G. 1998. Assessment of alternative soil management practices on N_2O emissions from US agriculture. *Agriculture Ecosystems & Environment*. 70: 79-87.

Paterson, A.H., Bowers, J.E., Bruggmann, R., Dubchak, I., Grimwood, J., Gundlach, H., Haberer, G., Hellsten, U., Mitros, T., Poliakov, A., Schmutz, J., Spannagl, M., Tang, H., Wang, X., Wicker, T., Bharti, A.K., Chapman, J., Feltus, F.A., Gowik, U., Grigoriev, I.V., Lyons, E., Maher, C.A., Martis, M., Narechania, A., Otillar, R.P., Penning, B.W., Salamov, A.A., Wang, Y., Zhang, L., Carpita, N.C., Freeling, M., Gingle, A.R., Hash, C.T., Keller, B., Klein, P., Kresovich, S., McCann, M.C., Ming, R., Peterson, D.G., Mehboob-ur-Rahman, Ware, D., Westhoff, P., Mayer, K.F., Messing, J. & Rokhsar, D.S. 2009. The *Sorghum bicolor* genome and the diversification of grasses. *Nature*. 457: 551-556.

Paterson, A.H., Schertz, K.F., Lin, Y.R., Liu, S.C. & Chang, Y.L. 1995. The weediness of wild plants - molecular analysis of genes influencing dispersal and persistence of Johnsongrass, *Sorghum halepense* (L) Pers. In *Proceedings of the National Academy of Sciences of the United States of America*. 92: 6127-6131.

Pimentel, D., Harvey, C., Resosudarmo, P., Sinclair, K., Kurz, D., McNair, M., Crist, S., Shpritz, L., Fitton, L., Saffouri, R. & Blair, R. 1995. Environmental and economic costs of soil erosion and conservation benefits. *Science*. 267: 1117-1123.

Piper, J.K. 1998. Growth and seed yield of three perennial grains within monocultures and mixed stands. *Agriculture Ecosystems & Environment*. 68: 1-11.

Randall, G.W. & Mulla, D. 2001. Nitrate nitrogen in surface waters as influenced by climatic conditions and agricultural practices. *Journal of Environmental Quality*. 30: 337-344.

Rooney, W.L., Blumenthal, J., Bean, B. & Mullet, J.E. 2007. Designing sorghum as a dedicated bioenergy feedstock. *Biofuels Bioproducts & Biorefining-Biofpr*. 1: 147-157. doi:10.1002/bbb.15.

Schauer, N., Semel, Y., Roessner, U., Gur, A., Balbo, I., Carrari, R., Pleban, T., Perez-Melis, A., Bruedigam, C., Kopka, J., Willmitzer, L., Zamir, D. & Fernie, A.R. 2006. Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. *Nature Biotechnology*. 24: 447-454.

Scheinost, P. 2001. Perennial wheat: a sustainable cropping system for the Pacific Northwest. *American Journal of Alternative Agriculture*. 16: 147-151.

Sobral, B.W.S., Braga, D.P.V., Lahood, E.S. & Keim, P. 1994. Phylogenetic analysis of chloroplast restriction enzyme site mutations in the Saccharinae Griseb subtribe of the Andropogoneae Dumort tribe. *Theoretical and Applied Genetics*. 87: 843-853.

Spangler, R., Zaitchik, B., Russo, E. & Kellogg, E. 1999. Andropogoneae evolution and generic limits in Sorghum (Poaceae) using ndhF sequences. *Systematic Botany*. 24: 267-281.

Swigonova, Z., Lai, J., Ma, J., Ramakrishna, W., Llaca, V., Bennetzen, J.L. & Messing, J. 2004. Close split of sorghum and maize genome progenitors. *Genome Research*. 14: 1916-1923.

Tanksley, S.D., Grandillo, S., Fulton, T.M., Zamir, D., Eshed, Y., Petiard, V., Lopez, J. & Beck-Bunn, T. 1996. Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative L-pimpinellifolium. *Theoretical and Applied Genetics*. 92: 213-224.

Tilman, D., Socolow, R., Foley, J.A., Hill, J., Larson, E., Lynd, L., Pacala, S., Reilly, J., Searchinger, T., Somerville, C., & Williams, R. 2009. Beneficial biofuels – the food, energy, and environment trilemma. *Science*. 325: 270-271.

Upadhyaya, H.D., Pundir, R.P.S., Dwivedi, S.L., Gowda, C.L.L., Reddy, V.G. & Singh, S. 2009. Developing a mini core collection of sorghum for diversified utilization of germplasm. *Crop Science*. 49: 1769-1780.

Wagoner, P. 1990. Perennial grain development – Past efforts and potential for the future. *Critical Reviews in Plant Sciences*. 9: 381-408.

Xiao, J., Li, J., Grandillo, S., Ahn, S.N., McCouch, S., Tanksley, S. & Yuan, L.P. 1996. A wild species contains genes that may significantly increase the yield of rice. *Nature*. 384: 223-224.

Zamir, D. 2001. Improving plant breeding with exotic genetic libraries. *Nature Reviews Genetics*. 2: 983-989.

08

BREEDING AND GENETICS OF PERENNIAL MAIZE:

PROGRESS, OPPORTUNITIES AND CHALLENGES

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ABSTRACT

Perennial crops have many hypothesized benefits (i.e. intercepted sunlight and nutrients over longer periods, increased yields, decreased soil erosion, improve stability of grain production, etc.) making them attractive for sustainable production. Annual maize (*Zea mays* L.), domesticated ~9 000 years ago, is one of the most important world crops and billions of dollars have been spent towards its improvement. However, there has been minimal effort to breed perennial maize and benefits hypothesized remain untested. Through crosses between annual maize and its two interfertile perennial maize relatives: tetraploid *Z. perennis* and diploid *Z. diploperennis*, maize germplasm and genomics knowledge can be leveraged to develop perennial maize. A few crosses and populations have been made by other investigators, notably Brewbaker, Carlson, Doebley, Holland, and Shaver but no adequate-yielding perennial maize has been developed. Compared with breeding perennial sorghum (a close maize relative) maize appears to have additional recessive quantitative traits that must be pyramided (senescence, tillering, perennial rhizomes/crowns, regrowth, heat and cold tolerance). I have now integrated the aforementioned perennial derived germplasm for nine breeding seasons with additional crosses to *Z. diploperennis* but very few to annual maize. My methodology has involved a cycle of 1) selfing crosses to purge deleterious recessive alleles; 2) selecting at the end of each season for non-senescence, regrowth/tillering and large ear size and 3) crossing between germplasm derived from different sources to pyramid complementary advantageous traits. Dramatic progress in adaptation, ear size, non-senescence and stability has been obtained, but this is confounded with evaluation environments. As of yet, we have not found a derived plant that oversummers, overwinters and produces rhizomes in Texas as the wild species do. A number of novel phenotypes have been observed including "ear forest", clumping grass types versus large tillered stalks, and prolificacy. A "one-best" ideotype for perennial maize still remains elusive.

Keywords: perennial, *Zea*, maize, sorghum, breeding, *Z. diploperennis*

INTRODUCTION

Nearly all domesticated grain crops are annuals that senesce and die after reproduction within the growing season in which they were planted, or they are managed as annuals, as in the case of sorghum, which is weakly perennial in warm climates. Perennials unlike our annual crop plants have an ability to overwinter and generally survive three or more successive years. Despite great successes in production, annual grain systems suffer from negative externalities including high input costs, soil erosion, and a limited growing season. Specific perceived economic and environmental benefits of perennial crops include increased vegetative cover over longer growing periods leading to increased photosynthate assimilation, decreased planting costs and reduction of energy-intensive inputs. Expanded root architecture of perennial crops could increase soil sequestration of carbon, further decrease off-season soil erosion, increase temporal access to water and nutrients among many other benefits. Perennial crops are one likely approach to improving sustainable food, feed, and fuel production with decreased land availability and access to inputs under a changing climate (Pimentel *et al.* 2012). Pastures and grasslands, which are generally managed as perennial systems, demonstrate improvements in critical ecosystem services; however, they do not produce grain needed for food, feed, and fuel uses. Despite these benefits the successful development of perennial grain crops has been sparse and largely limited to a few C3 grasses such as rice (Sacks *et al.* 2003, 2006), wheat (Scheinost *et al.* 2001; Murphy *et al.* 2009), and intermediate wheatgrass 'Kernza' (Cox *et al.* 2002; Strand 2010).

Among the productive and widely grown grain crops that humans have domesticated is maize (*Zea mays* L.). Genetic/breeding and agronomic improvement of maize production in the United States of America and the rest of the developed world has been incredibly successful, increasing production as much as 800 percent from a century ago; meaning eight times less land is needed to produce the same amount of grain (Brummer *et al.* 2011). While yields continue to increase at 1-2 percent in areas of the Midwestern United States of America with the heaviest investment, they have remained stable over the last 10 years in lower yielding and lower investment areas such as Texas (Barerro *et al.* 2013). In the developing world, yields have also not increased at the same rate, which is likely due to both lack of agronomic inputs (fertilizer and irrigation) and limited genetic improvement of the crop. Maize could provide the most immediate impact from the development of perennial cultivars if these cultivars would maintain or increase productivity while providing improved ecosystem services and/or decreased costs of production and allow mitigation and improved adaptation to climate change.

PERENNIALISM ACROSS THE GRASSES

From a comparative breeding, physiology and developmental perspective, it is important to note that all C4 grass crops have close perennial relatives within their genus or are perennial themselves. Sorghum (*Sorghum bicolor* L. Moenech) is an important grain, forage, sugar and bioenergy crop grown as an annual that is also closely related to maize. *Sorghum propinquum* and *Sorghum halepense* (also known as Johnsongrass) are both perennial. Close relative Sugarcane (*Saccharum officinarum* L.), is already grown and managed as a perennial. Close relative pearl millet (*Pennisetum glaucum*) has perennial relative Napier grass (*Pennisetum purpureum)*. Napier grass, miscanthus (*Miscanthus giganteus)*, switchgrass (*Panicum virgatum*) and giant reed (*Arundo donax* L.) are undergoing domestication and improvement for forage, restoration, and bioenergy systems specifically because they are perennial (Lewandowski *et al.* 2003; Jessup 2009). Generating and synthesizing information from across all of these species will likely yield the most fruitful understanding of improving perennial phenotypes. Among these species, *Zea* and *Sorghum* provide the most complementary applied and research targets for perennialism with their wild relatives. Maize and sorghum are the 1st and 5th most important cereal crops worldwide, respectively (FAOSTAT, 2013). These crops also have active breeding and genetics programs throughout the world and have extensive genomic information available. Genome annotations have been completed for *Z. mays* (Schnabel *et al.* 2009; Rokhsar *et al.* 2009; Vielle-Calzada *et al.* 2009) and *S. bicolor* (Paterson *et al.* 2009). Because little is known of the molecular mechanisms conditioning the annual/perennial divide across grass species and given the complexity of the trait, molecular dissection would likely be fruitful.

WHAT IS PERENNIAL MAIZE?

Maize has two perennial relatives in its genus; *Zea perennis* (tetraploid – Mexico), *Z. diploperennis* (diploid –Mexico). One of the first reports of crosses within a *Zea* perennial species was that of Emerson and Beadle (1929) who crossed maize to what we now know as *Z. perennis*. Mangelsdorf and Reeves (1939) went further in the investigations of multiple maize crosses with *Z. perennis* and eastern gamagrass, a temperately-adapted, perennial native grass in the genus *Tripsacum* that resides in the tertiary gene pool of maize. Shaver (1964) was one of the first to seriously investigate the possibility of creating perennial domesticated maize. Shaver continued Emerson and Beadles populations and also crossed *Z. perennis* with colchicine doubled maize to investigate the genetic inheritance. From these crosses, Shaver derived perennial 4N (tetraploid) and 2N (diploid through pollen sieving) populations. The 2N, however, appeared to nearly lose the perennial phenotype, likely because of the complex suite of genes/alleles needed. The 4N and 2N populations have now been selected for over 20 generations each and have been shared with many other investigators (Shaver, pers. comm.). While many investigators have given up on these populations, a few have gone on to report interesting traits such as nodal propagation (Ting and Tran, 1999). Nodal propagation is a trait clearly possible with perennial sugarcane, which is how cultivars of sugarcane are propagated, but not possible in annual maize.

In 1979, Iltis *et al.* reported a new diploid perennial *Z. diploperennis* had been discovered and since that time it has been crossed by only a few other investigators and generally not for perennialism (Srinivasan and Brewbaker, 1999). J. Holland successfully crossed *Z. diploperennis* to B73 (the maize reference line with the sequenced genome) and NC300 (an elite inbred line important in the southern United States). F_2 progeny of these crosses were investigated both in North Carolina by Holland and by Murray in College Station and Weslaco, Texas. Both Holland and Murray found that combining with B73 was quite poor compared with NC300 in hybrid combination. In Weslaco, the F_1 and F_2 were crossed to late flowering elite Texas maize lines and were also allowed to open pollinate among themselves. From this, 250 progeny were planted in College Station, TX in 2009 and those that demonstrated at least one perennial type trait, (indeterminacy/staygreen, tillering, etc.; described later) were self-pollinated. Shaver's 2N (diploid) and 4N perennial populations (tetraploid) were also late planted in College Station in 2009 and demonstrated a range of perennial phenotypes but due to extreme summer and winter conditions none were found to overwinter. From Shavers populations out of ~5000 2N plants and ~5000 4N plants, 99 desirable plants from 2N were pollinated, and 34 from 4N were pollinated and planted ear to row in 2010. Plants in this population have been primarily sib-mated but also self-pollinated, crossed with the Holland derived material and crossed with perennial wild relatives by Murray now for a total of nine additional seasons. Germplasm generated and selected has greatly improved for ear size and grain yield which is critical for perennial seed and grain production. A range of other phenotypes has been observed to improve including tillering, totipotency of stem tissue and delayed or eliminated senescence. However, no rhizomes have been observed and plants left in the field have generally died from drought, heat or freezing. Meanwhile, multiple wild *Z. diploperennis* plants have survived for over four years. This continues to suggest that perennial maize could be developed through incorporation of key genes but more work is needed.

GENETICS OF PERENNIALISM

One QTL mapping study of 425 F_2 plants derived from *Z. diploperennis* crossed to the annual teosinte *Z. mays* ssp. *parviglumis* has been reported that used 95 RFLPs (Westerbergh and Doebley, 2004). Very few of the plants had rhizomes and only two QTL for rhizomes were identified but explained less than 12 percent of the variation. They attributed this low amount of variation to low trait heritability as we might expect in an F_2 study of a threshold trait where the phenotype of interest needs a suite of genes and a favourable environment for screening. However, these findings compare poorly to those in sorghum. Paterson *et al.* (1995) reported a mapping study of 370 F_2 and 378 BC_1 plants in a cross between *S. bicolor* × *S. propinquum* with 78 RFLPs. In this study many more plants had rhizomes and 10 QTL were detected explaining 48 percent of the variance. These findings between perennial maize and perennial sorghum dissection are very consistent with my personal observations having worked with both species. Both the rhizomes and the perennialism observed in *Sorghum* are much more robust than that in *Zea*.

What do we need to make maize perennial?

For a maize plant to act as a perennial a number of conditions must be met: the plant must not senesce at the end of the season; the plant must accumulate energy into structures that can overwinter; the plant must be able to prevent its overwintering structures from both freezing and degradation; and finally the plant must remobilize energy from the overwintering structures into new regrowth in the spring. For perennial maize to actually be grown by farmers this must all be accomplished in a plant that can produce adequate grain yields and can switch back and forth between reproductive and vegetative growth – in addition to the other suites of traits that farmers desire such as disease resistance, nutrient and water use efficiency. The breeding progress for selection on these many different quantitative traits is certain to be slow, but I feel all must be selected simultaneously to avoid breeding into a corner.

There are many quantitative tendencies referred to as perennial-like that do not necessarily result in overwintering. First, stay green, or delayed senescence; from evidence in grain sorghum and maize this is an important trait to maximize grain filling under stress (Thomas and Howarth, 2000; Campos *et al.* 2004). Progeny of perennial by domesticated crosses often show much greater stay green. However, even the most staygreen types of commercial maize senesce completely at the end of grain filling and senescence will be the most important trait to select against in perennial x domesticated *Zea* crosses. Some progeny have had a deceiving staygreen appearance that seems to be due to delayed flowering time and maturity. Domesticated annual sorghum does not naturally senesce and does not appear to be a limiting trait for a perennial phenotype, as many researchers already consider sorghum a weak non-overwintering perennial. This may be the cause of the more heritable perennialism in sorghum. Second, the ability to tiller, ratoon or produce additional stalks from the crown is likely an important component to perennialism. However, basal tillering is also found in many landrace maize cultivars, sweet corn, and many grasses including most annual sorghum; therefore Shaver (1964) cautions against using tillering as a proxy selection criteria alone for perennialism in maize. It has been my observation that often tillering is an underground branching not specifically related to active meristem tissue. Third, ratooning or the ability to regrow tillers when the main stalk is cut is a phenotype expected from perennial plants. However, annual maize and many types of sorghum harvested green for silage can also demonstrate ratooning ability under the rare proper environmental conditions (Coors *et al.* 2007; Livingston and Coffman, 1997). These annual ratooning plants, however, are not able to overwinter because they often senesce and also do not produce rhizomes. Fourth, the ability to produce rhizomes, modified underground storage stems that allow vegetative reproduction, is an important and heritable phenotype that is found in many perennial plants but no annual ones. Rhizomes are totipotent, can overwinter underground and produce new plantlets (rammets) in the same seasons or in following years from nodes that grow tillers. This totipotency of rhizomes makes them important in perennial growth habit. Perennialism in *S. halepense* (Johnsongrass) has been found to require rhizomes and to be primarily, but not solely, a function of rhizome depth (Warwick *et al.* 1987; Washburn 2012). While the goal is to produce a high-yielding perennial crop and not a noxious weed difficulty killing vigorous regrowth alone is not a desired criterion. As an alternative to rhizomes, many temperate plants (switchgrass, cordgrass, bahiagrass, etc.) regrow from the crowns via carbohydrate reserves in short, knotty proaxes occasionally mistaken for rhizomes (Haferkamp and Copeland, 1984; Boe *et al.* 2009). This is the overwintering mechanism in close *Zea* relative eastern gamagrass (*Tripsacum dactyloides* L.) (Jackson and Dewald, 1994), its interspecific hybrids with maize (Jatimliansky *et al.* 2004). This may also be the case for *Z. diploperennis*, in which rhizomes are clearly but only occasionally produced. Regrowth from the crown proaxes in combination with rhizomes would provide an avenue for developing robust perennial maize with multiple overwintering mechanisms. Finally, overwintering, which is the most important component of true perennials will be conditioned by all of these traits and their interaction with the environment. Because of this complexity perennialism is a threshold trait, or one that if even one component or gene/allele is missing, or if the material is screened in a poor environment, we will not be able to observe any plants as perennial. Selection on a complex quantitative trait like perennialism will not be as efficient as selection on a simple additive trait. In both maize and in sorghum we have already developed and/or evaluated genetic resources that improve the chances of rescuing a perennial plant.

CHALLENGES AND INTERESTING TRAITS

While perennialism is the primary goal of this research, a number of other traits of interest have arisen in the germplasm that could be useful for farmers, environment or society. *Later canopy cover* – At the end of an annual maize growing season, not only the nutrients but also the sunlight is typically striking bare ground and not being turned into useful biomass for humans or agro-ecosystems. We observe that the perennial derived material has much greater green leaf tissue at the end of the season, whether through branching, tillering, and/or delayed senescence. *Delayed/non-senescence* – the wild species and many of the crosses do exhibit staygreen or delayed senescense, this is believed to be associated with increased stress tolerance, grain filling period and increased yield. Many breeders look for increased staygreen, especially in hot, arid, tropical climates. *Prolificacy* – the ability to produce many ears on the same plant has been a long selected trait by maize breeders. These plants tend to produce ears under even the most stressful condition. Many plants derived in the perennial maize crossing program have multiple ears on each stalk or multiple stalks with at least one ear. *Indeterminant regrowth* – the ability to produce biomass after initial flowering and seed set would be very advantageous for making the most of environmental resources. This biomass would be useful for preventing soil erosion, capturing carbon, and providing additional silage or cellulosic biofuel feedstocks. *Ear forest* – some of the germplasm continues to produce a succession of ears that flower at the base of the plant until drought, heat or freeze kills the plant. A potential application for this trait is to graze livestock that can digest starch. *Deeper rooting* – We observe the root systems of perennial *Zea* to typically be much larger and deeper than annual maize. This suggests that they can access additional water and nutrients and may contribute additional biomass to the soil. *Increased sinks* – In the case of sorghum and sugarcane it has been clearly shown that increased diversity of sink tissue results in an increase of potential harvestable energy and that "tradeoffs" are unlikely to be a problem if cultivars are selected for multiple sinks (Murray *et al.* 2012). This is in large part because photosynthesis has been shown to be sink as opposed to source limited.

CONCLUSIONS

The domestication of crops was a long-term proposition and it has taken over 100 years and billions of research dollars to get maize to reach its current productivity. Developing high-yielding perennial maize is likely to take an additional 10 to 40 years, at which point hypotheses of yield and eco-system service comparisons can be formally tested. This long-term high risk research is most appropriate for the public sector. This is concerning given the erosion of public research funds, especially in agriculture. Substantial commitments and investments will be needed to make perennial maize a reality.

FIGURE 1. *ZEA DIPLOPERENNIS*

Fig1A-B: Division of part of a *Z. diploperennis* rhizome from a pot.
Fig1C: Appearance of *Z. diploperennis* in late summer / fall (College Station, 2010). This has not started flowering while everything else is long dead.

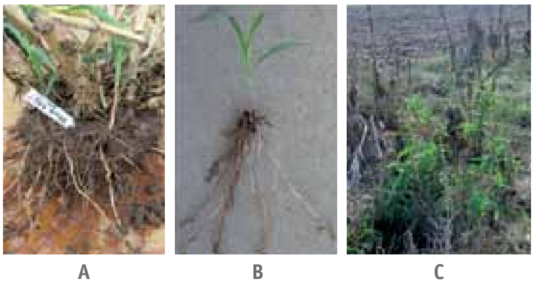

FIGURE 2. DIFFERENCES IN ROOTS

Weslaco, December 2013

REFERENCES

Barerro, I.D.F., Murray, S.C., Pietsch, D. & Labar, S. 2013. A multi-environment trial analysis of commercial maize shows a slight grain yield improvement in Texas. *Field Crops Research*. 149:167-176.

Boe, A., Owens, V., Gonzalez-Hernandez, J., Stein, J., Lee, D.K. & Koo, B.C. 2009. Morphology and biomass production of prairie cordgrass on marginal lands. *GCB Bioenergy*. 1:240-250.

Brummer, E.C., Barber, W.T., Collier, S., Cox, T.S., Johnson, R., Murray, S.C., Olsen, R.T., Pratt, R.C. & Thro, A.M. 2011. Plant breeding for harmony between agriculture and the environment. *Frontiers of Ecology and the Environment*. 9:561-568.

Campos, H., Cooper, M., Habben, J.E., Edmeades, G.O. & Schussler, J.R. 2004. Improving drought tolerance in maize: a view from industry. *Field Crops Research*. 90: 19-34.

Coors, J.G., Eilert, D.T. & Flannery, P.J. 2010. Plants and seeds of corn comprising brown midrib and gt1 genes. U.S. Patent No. 7,723,584. Washington, DC: U.S. Patent and Trademark Office.

Cox, T.S., Bender, M., Picone, C., Van Tassel, D.L., Holland, J.B., Brummer, E.C., Zoeller, B.E., Paterson, A.H., & Jackson, W. 2002. Breeding Perennial Grain Crops. *Critical Reviews in Plant Sciences*. 21: 59-91.

Davis, J.G. & Edye, L.A. 1959. *Sorghum almum* Parodi, a valuable summer growing grass. *Journal of the Australian Institute of Agricultural Science*. 25: 117-27.

Emerson, R.A. & Beadle, G.W. 1930. A fertile tetraploid hybrid between *Euchlaena perennis* and *Zea mays*. *American Naturalist*. 64:190-192.

FAOSTAT. 2013. *ProdSTAT*. (Available at http://faostat.fao.org/site/567/DesktopDefault.aspx#ancor).

Haferkamp, M.R. & Copeland, T.D. 1984. Shoot growth and development of Alamo switchgrass as influenced by mowing and fertilization. *Journal of Range Management*. 37: 406-412.

Iltis, H.H., Doebley, J.F.R., Guzman, M. & Pazy, B. 1979. *Zea diploperennis* (Gramineae): A new teosinte from Mexico. *Science*. 203: 186-188.

Jackson, L.L. & Dewald, C.L. 1994. Predicting evolutionary consequences of greater reproductive effort in *Tripsacum dactyloides*, a perennial grass. *Ecology*. 75: 627-641.

Jatimliansky, J.R., Garcia, M.D. & Molina, M.C. 2004. Response to chilling of *Zea mays, Tripsacum dactyloides* and their hybrid. *Biologia Plantarum*. 48: 561-567.

Jessup, R.W. 2009. Development and status of dedicated energy crops in the United States. *In Vitro Cellular & Developmental Biology-Plant*. 45:282-290.

Lewandowski, I., Scurlock, J.M.O., Lindvall, E. & Christou, M. 2003. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass and Bioenergy*. 25: 335-361.

Livingston, S. & Coffman, C.G. 1997. Ratooning grain sorghum on the Texas gulf coast. *Texas Agricultural Extension Service Bulletin*. M—8-96. (Available at http://publications.tamu.edu/CORN_SORGHUM/PUB_Ratooning%20Grain%20Sorghum%20on%20the%20Texas%20Gulf%20Coast.pdf).

Mangelsdorf, P.C. & Reeves, R.G. 1939. The origin of Indian corn and its relatives. Vol. 574. *Agricultural and Mechanical College of Texas*.

Murray, S.C. 2012. Differentiation of seed, sugar, and biomass-producing genotypes in Saccharinae species. In Paterson, A.H. ed. Springer. p. 479-502.

Paterson, A.H., Schertz, K.F., Lin, Y.R., Liu, S.C. & Chang, Y.L. 1995. The weediness of wild plants:Molecular analysis of genes influencing dispersal and persistence of johnsongrass, *Sorghum halepense* (L.) Pers. *Proceedings of the National Academy of Sciences*. 92: 6127-6131.

Paterson, A.H., Bowers, J.E., Bruggmann, R., Dubchak, I., Grimwood, J., Gundlach, H., Haberer, G., Hellsten, U., Mitros, T., Poliakov, A., Schmutz, J., Spannagl, M., Tang, H., Wang, X., Wicker, T., Bharti, A.K., Chapman, J., Feltus, F.A., Gowik, U., Grigoriev, I.V., Lyons, E., Maher, C.A., Martis, M., Narechania, N., Otillar, R.P., Penning, B.W., Salamov, A.A., Wang, Y., Zhang, L., Carpita, N.C., Freeling, M., Gingle, A.R., Hash, C.T., Keller, B., Klein, P., Kresovich, S., McCann, M.C., Ming, R., Peterson, D.G., Rahman, M., Ware, D., Westhoff, P., Mayer, K.F.X., Messing, J. & Rokhsar, D.S. 2009. The *Sorghum bicolor* genome and the diversification of grasses. *Nature*. 457: 551-557.

Pimentel, D., Cerasale, D., Stanley, R.C., Perlman, R., Newman, E.M., Brent, L.C., Mullan, A. & Changa, D.T-I. 2012. Annual vs. perennial grain production. *Agriculture, Ecosystems and Environment*. 161: 1-9.

Rokhsar, D., Chapman, J., Mitros, T. & Goodstein, D. 2009. Update on the Mo17 genome sequencing project. *Maize Genetics Conference Abstracts*. 51:(T) 13.

Sacks, E.J., Roxas, J.P. & Cruz, M.T.S. 2003. Developing perennial upland rice I: field performance of *Oryza sativa/O. rufipogon* F1, F4 and BC1F4 progeny. *Crop Science*. 43: 120-128.

Sacks, E.J., Dhanapala, M.P., Tao, D.Y., Cruz, M.T.S. & Sallan, R. 2006. Breeding for perennial growth and fertility in an *Oryza sativa/O. longistaminata* population. *Field Crops Research*. 95: 39-48.

Scheinost, P.L., Lammer, D.L., Cai, X., Murray, T.D. & Jones, S.S. 2001. Perennial wheat: the development of a sustainable cropping system for the U.S. Pacific Northwest. *American Journal of Alternative Agriculture*. 16: 147-151.

Schnable, P.S., Ware, D., Fulton, R.S., Stein, J.C., Wei, F.S., Pasternak, S., Liang, C., Zhang, J., Fulton, L., Graves, T.A., Minx, P., Reily, A.D., Courtney, L., Kruchowski, S.S., Tomlinson, C., Strong, C., Delehaunty, K., Fronick, C., Courtney, B., Rock, S.M., Belter, E., Du, F., Kim, K., Abbott, R.M., Cotton, M., Levy, A., Marchetto, P., Ochoa, K., Jackson, S.M., Gillam, B., Chen, W., Yan, L., Higginbotham, J., Cardenas, M., Waligorski, J., Applebaum, E., Phelps, L., Falcone, J., Kanchi, K., Thane, T., Scimone, A., Thane, N., Henke, J., Wang, T., Ruppert, J., Shah, N., Rotter, K., Hodges, J., Ingenthron, E., Cordes, M., Kohlberg, S., Sgro, J., Delgado, B., Mead, K., Chinwalla, A., Leonard, S., Crouse, K., Collura, K., Kudrna, D., Currie, J., He, R., Angelova, A., Rajasekar, S., Mueller, T., Lomeli, R., Scara, G., Ko, A., Delaney, K., Wissotski, M., Lopez, G., Campos, D., Braidotti, M., Ashley, E., Golser, W., Kim, H., Lee, S., Lin, J., Dujmic, Z., Kim, W., Talag, J., Zuccolo, A., Fan, C., Sebastian, A., Kramer, M., Spiegel, L., Nascimento, L., Zutavern, T., Miller, B., Ambroise, C., Muller, S., Spooner, W., Narechania, A., Ren, L., Wei, S., Kumari, S., Faga, B., Levy, M.J., McMahan, L., Van Buren, P., Vaughn, M.W., Ying, K., Yeh, C-T., Emrich, S.J., Jia, Y., Kalyanaraman, A., Hsia, A-P., Barbazuk, W.B., Baucom, R.S., Brutnell, T.P., Carpita, N.C., Chaparro, C., Chia, J-M., Deragon, J-M., Estill, J.C., Fu, Y., Jeddeloh, J.A., Han, Y., Lee, H., Li, P., Lisch, D.R., Liu, S., Liu, Z., Nagel, D.H., McCann, M.C., SanMiguel, P., Myers, A.M., Nettleton, D., Nguyen, J., Penning, B.W., Ponnala, L., Schneider, K.L., Schwartz, D.C., Sharma, A., Soderlund, C., Springer, N.M., Sun, Q., Wang, H., Waterman, M., Westerman, R., Wolfgruber, T.K., Yang, L., Yu, Y., Zhang, L., Zhou, S., Zhu, Q., Bennetzen, J.L., Dawe, R.K., Jiang, J., Jiang, N., Presting, G.G., Wessler, S.R., Aluru, S., Martienssen, R.A., Clifton, S.W., McCombie, W.R., Wing, R.A. & Wilson R.K. 2009. The B73 maize genome: complexity, diversity, and dynamics. *Science*. 326: 1112–1115.

Shaver, D.L. 1964. Perennialism in *Zea*. *Genetics*. 50: 393-406.

Srinivasan, G. & Brewbaker, J.L. 1999. Genetic analysis of hybrids between maize and perennial teosinte. I. Morphological traits. *Maydica*. 44: 353-369.

Strand, M. 2010 Going with the grain. *The Salina Journal*. 10/1/2010. (Available at www.salina.com/news/story/wes-wheat).

Ting, Y. & Tran, L. 1999. Regeneration of plantlets in diploid maize. *Maize Genetics Newsletter*. 73:22

Thomas, H. & Howarth, C.J. 2000. Five ways to stay green. *Journal of Experimental Botany*. 51: 329-337.

Vielle-Calzada, J.P., de la Vega, O.M., Hernández-Guzmán, G., Ibarra-Laclette, E., Alvarez-Mejía, C., Vega-Arreguín, J.C., Jiménez-Moraila, B., Fernández-Cortés, A., Corona-Armenta, G. & Herrera-Estrella, L. 2009. The Palomero genome suggests metal effects on domestication. *Science*. 326: 1078-1078.

Warwick, S.I., Phillips, D. & Andrews, C. 1986. Rhizome depth: the critical factor in winter survival of *Sorghum halepense* (L.) Pers. (Johnson grass). *Weed Research*. 26: 381-387.

Washburn, J.D., Murray, S.C., Burson, B.L., Klein, R.R. & Jessup, R.W. 2013a. Targeted mapping of QTL regions for rhizomatousness in chromosome SBI-01 and analysis of overwintering in a *Sorghum bicolor* x *S. propinquum* population. *Molecular Breeding*. 31: 153-162.

Washburn, J.D., Whitmire, D.K., Murray, S.C., Burson, B.L., Wickersham, T.A., Heitholt, J.J. & Jessup, R.W. 2013b. Estimation of rhizome composition and overwintering ability in perennial *Sorghum* spp. using Near-Infrared Spectroscopy (NIRS). *Bioenergy Research*. DOI 10.1007/s12155-013-9305-8

Westerbergh, A. & Doebley, J. 2004. Quantitative trait loci controlling phenotypes related to the perennial versus annual habit in wild relatives of maize. *Theoretical and Applied Genetics*. 109: 1544-1553.

09

EVALUATING PERENNIAL CANDIDATES FOR DOMESTICATION:

LESSONS FROM WILD SUNFLOWER RELATIVES

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ABSTRACT

Recurrent selection for increased seed production has been successful in two wild perennial species related to sunflower, *Helianthus maximiliani* and *Silphium integrifolium*. A third species, *Helianthus pauciflorus* has highly heritable variation for seed yield and should respond to selection. Each wild species has a different set of traits that limit its usefulness as a grain crop and each has unique adaptations beyond the perennial habit that could enhance its value or accelerate its domestication. For example, *S. integrifolium* is deeply rooted and relatively unaffected by short term droughts, while *H. pauciflorus* is easily asexually propagated by tubers making clonal cultivars feasible.

More generally, we ask which traits make a wild perennial more genetically and agronomically tractable and which complex but ecologically important traits might only be brought to the agro-ecosystem through domestication? Any perennial grain reduces the need for tillage, but perennials are not equally drought tolerant, competitive with weeds or carbon sequestering. We suggest, from both experience and deduction, that seed yield and the classic domestication traits such as shattering may often be less important in selecting perennial species for domestication than genetically complex traits such as growth rate, phenology or branching pattern.

Keywords: perennial grains, oil crops, oilseeds, domestication, landscape genetics, crop wild relatives, functional traits, *Silphium, Helianthus, Linum*

INTRODUCTION

Perennial grains, including perennial cereals, pulses and oilseeds, are being investigated or developed at several institutions as a long-term solution to the ongoing problem of soil erosion and degradation associated with annual grain cropping systems. The rationale for increasing the proportion of agricultural land planted to perennial crops has been more fully articulated by Glover *et al.* 2010; while the potential physiological and genetic obstacles to combining high grain yield and multi-year lifespan—and suggestions for how these obstacles can be overcome-have also been explored elsewhere (DeHaan *et al.* 2007; Van Tassel *et al.* 2010). In this volume given this rationale we assume that high-yielding perennial oilseeds are both beneficial and possible to develop. We provide a rationale for investing in new oilseed crops in addition to the more obvious cereals and for choosing domestication in these cases instead of using wide hybridization to “perennialize” an existing crop. Next we survey the native North American species being domesticated as perennial oilseeds. We report progress and observations with these species, and describe our plans for continued domestication. Finally, we compare the candidates and look for principles that could help future domesticators identify candidates likely to respond rapidly to domesticating selection from among the many seed-bearing wild species.

I. Rationale

Perennial oilseed grains will contribute to long-term food security and renewable energy. Oil crops contribute to food security in every region because they are less expensive than animal fats and dietary lipids supply both calories and the essential fatty acids alpha-linolenic acid (an omega-3 fatty acid) and linoleic acid (an omega-6 fatty acid) that humans cannot synthesize. Furthermore, some fat in the diet greatly improves the bioavailability of at least one vitamin precursor, B-carotene (van het Hof *et al.* 2000), and could increase the uptake of other fat-soluble nutrients such as lycopene, lutein, Vitamins K and D (Brown *et al.* 2004).

Oilseed crops play an important role in the diversity of cropping systems, providing functional diversity to compliment grain and legume rotations. Many oilseeds are duel or multi-purpose crops, filling multiple end uses (Vollamann and Rajcan, 2010). For instance crops such as sunflower, soybean and canola provide human nutrients, protein-rich meal for animal consumption, and the raw feedstock for biofuels (Agarwal, 2007) and bioplastics and other industrial products (Xia and Larock, 2010) which can aid in reducing the dependency of petroleum based fossil fuels.

Perhaps the most compelling argument in favour of perennial oilseed research is simply that oil crops currently occupy 281 million hectares (FAOstat, 2012 data). This is about 26 percent of the world's acreage devoted to staple crops (cereals, pulses, oilcrops and roots/tubers) and about 18 percent of all land classified as arable or under permanent crops (FAOstat, 2011 data). For whatever reasons, humanity has chosen to devote a large proportion of the planet's land area to oil crops and providing more perennial oil crop options could reduce the ecological disservices associated with annual oil crops. Encouragingly, 41 percent of all vegetable oil already comes from perennials such as olive, oil palm, and coconut (FAOstat, 2012 data).

However, these perennial oil crops are all tropical or subtropical tree-like species and virtually all the oil crops in temperate regions are annuals such as soybean, sunflower, flaxseed and canola. Furthermore, tree-like crops are slow to establish and expensive to harvest and immobilize moisture and nutrients from the environment for extended periods. Perennial oilseed forbs could provide additional options even in the tropics, supplying some of the ecosystem services of the tree crops, as well as new services, such as fodder, and new harvesting and management (mowing, grazing and burning) options.

In both tropical and temperate regions, the development of perennial oilseeds, particularly those with diverse end-uses and capable of producing on marginal lands where annual cropping is at a high risk of failure, has the potential to increase the productivity of global agricultural systems.

Domestication of perennial oilseeds, perennialization of domestic oilseeds and intermediate approaches. There are currently four main routes to combining the yield of an annual crop with the longevity of a wild perennial. The first three require wide hybridization between an annual species and a perennial species. Under the "triticale option", a full or partial amphiploids with whole chromosomes of both species is created. If the chromosome constitution remains stable in successive generations, a new species has been created. Option 2 utilizes introgression breeding to move "domestication genes" from the cultigen into the wild perennial background. Option 3 is similar, but aims to move "perennialism genes" from the wild species into the genetic background of the cultivated species. The fourth option—domestication—attempts to find rare alleles in the primary gene pool of a wild perennial that confer favourable "domestication syndrome" traits such as reduced shattering, increased threshability, etc.

All of these routes are being attempted with members of the sunflower family. Researchers at The University of Minnesota (Hulke and Wyse, 2008; Kantar *et al.* 2010; Kantar *et al.* 2014) made wide hybrids between *Helianthus tuberosus* and the crop sunflower (*Helianthus annuus*) followed by using the annual (diploid or tetraploid lines) as the recurrent parent. Two of us (Van Tassel and Cox) have attempted to select for stable amphiploids from among the progeny of intermated wide hybrids between either *Helianthus tuberosus* or *H. pauciflorus* (perennials) and the crop sunflower. While the details of these programs are beyond the scope of this report, the summary is that stable amphiploids are very rare and exhibit low fertility. Even if found, these lines are unlikely to contain the allelic diversity needed for long-term crop improvement and cultivar development. Additional amphiploids of the same karyotype will have to be developed for that. Similarly, crossing hybrids with either the annual or perennial parent often appears to result in the elimination of many or all of the chromosomes of one species or the other.

The situation appears to be very similar to that encountered when trying to transfer genes from *Tripsacum* to *Zea*. Harlan and DeWet (1977) wrote:

The cytogenetic interactions in maize x *Tripsacum* hybrid derivatives can be remarkably complex. The number of viable, somewhat female fertile chromosome combinations is astonishing. Fifty-four different combinations are listed in this paper, and others are certainly possible. In successive backcrosses to maize, chromosome numbers scale both up and down with ease, sometimes stabilizing for a few generations via apomixis, but in most cases finally generate balanced maize genomes and emerge as recovered maize. The maize, however, can produce strange and unusual phenotypes as a result of genetic transfers from *Tripsacum*. Highly tripsacoid maize lines with 2n = 20 chromosomes were recovered in pathways where the maize genome had first contaminated the *Tripsacum* genome in early backcross generations. In other pathways interaction of the two genomes is rare.

Cox is currently attempting to develop “annuus-oid” *H. pauciflorus* by making numerous F1 hybrids between *H. pauciflorus* and *H. annuus*, using the perennial as the female and then “backcrossing” the F1s to the perennial until a fertile, balanced *H. pauciflorus* genome is recovered. Her aim is to introgress a few “domestication genes” into *H. pauciflorus*, and her hypothesis is that even a few alien alleles increasing seed or head size could greatly accelerate the domestication of *H. pauciflorus*. It seems to these authors that introgressing the perennial habit into the annual background is a more daunting task because several to many alien genes are likely to be required simultaneously in order to produce a perennial and winter-hardy phenotype.

Domestication: slow but steady. In contrast with wide hybridization, fertile parents in a domestication population are intermated and fertile offspring are produced which can be easily intermated, crossed with new, wild individuals, or backcrossed to their parents. The “astonishing” number of cytotypes and dramatic “strange and unusual” phenotypes that Harlan and DeWet (1977) saw with the derivatives of interspecific hybrids and that Van Tassel and Cox have seen with interspecific Helianthus are not generated by this approach. There is no possibility of making a single cross that fortuitously combines all the desirable traits of the domestic species and the longevity and hardiness of the perennial. But, on the other hand, serious problems with sterility, chromosome number fluctuation and development of small populations with strong barriers to gene flow with other sources of genetic variation are unlikely to be encountered. Domestication is a slow and steady strategy unlikely to produce dramatic "early wins" but also unlikely to fail if given enough time. In experimental populations, recurrent phenotypic selection on individuals has repeatedly resulted in the evolution of novel phenotypes (e.g. Ratcliff *et al.* 2012; reviewed by Travisano and Shaw, 2013).

Recombination load is the reduction of fitness caused by breaking favourable genetic associations that have been generated by selection (Otto and Lenormand, 2002). Presumably the compliment of chromosomes in a genome represents one of the strongest forms of genetic association. The addition of entire or large pieces of alien chromosomes is almost certain to disrupt the previous genetic associations. Beyond disrupting the process of meiosis itself, wide crossing recombines whole developmental programs sometimes leading to misshapen, fragile or non-functional organs (Siikamaki, 1999). Any adaptations involving whole plant architecture and/or finely tuned phenology are likely to be lost following wide hybridization. On the other hand, the crop field may represent such a novel environment that reductions in fitness in the wild environment are irrelevant.

Domestication as a source of new, complex and functional traits for the farm. The wild relatives of crops can be little more than genetic resources to be mined for novel alleles when only a few genes are likely to be successfully transferred to the domestic species by either introgression or transgenics. However, through domestication, entire biochemical pathways, plant organs or life history strategies are available.

Conceptually treating all domesticated grain species as a single ecological guild we can draw on the emerging field of landscape genetics (Manel *et al.* 2010) to suggest the value of dramatically increasing the genetic and phenotypic variation within grain crops by adding new species with new complex traits. Landscape variables play a vital role in conventional plant breeding as genotype x environment interactions determine the suitability of a particular crop or cultivar to a given region. While the aim of population genetics, a cornerstone of plant breeding, is the study of allele frequencies under various adaptive and neutral processes (Falconer and MacKay, 1996), landscape ecology aims to characterize the relationship between various environmental ecological processes and ecosystems (Ewers *et al.* 2013). The discipline of landscape genetics aims to examine how specific geographic and environmental factors shape population structure and can be useful in identifying traits of ecological significance. By extension ecological genetics is the specific study of these traits of ecological significance (Lesica and Allendorf, 1999) some of which are of interest to plant breeders.

Grain crop traits related to adaptation to novel habitats and abiotic stresses such as flooding and drought stress are increasingly sought after by plant breeders for the maintenance of yield in marginal or unpredictable environments. An ecological genetics approach may force breeders to look outside of conventional selection criteria because the domestication and later crop improvement of our current crops occurred under a particular set of "idealized" conditions; including growing regions with favourable rainfall or irrigation, fertile valley soils, monoculture cropping, and weed control. This process is inherently selective for genotypes—and species--that do well under these conditions and not necessarily marginal landscapes. Many wild species on the other hand have undergone selection under extreme environmental conditions that do not normally present themselves in ideal agricultural systems and provide novel materials for crop development (Vear, 2010).

Domestication can now be accelerated. Advances in sequencing technology and statistical modelling are starting to eliminate barriers to the genetic dissection of complex quantitative and plastic ecological traits even in species which lack genomic resources or known pedigree (Sillanpää, 2011; Davey *et al.* 2011; Baxter *et al.* 2011; Narum *et al.* 2013). While important ecological traits such as nitrogen or water use efficiency may be the result of a combination of many genes each contributing small portions to the total variance in the trait throughout the genome, high molecular marker saturation is allowing for the development of whole genome breeding values and GS for complex traits using whole genome marker saturation. Whole genome GS using markers development techniques such as genotype-by-sequencing and RAD-seq have been effective in predicting selection candidates for complex traits such as grain yield in wheat (Poland *et al.* 2012) and are becoming more common in both plant breeding and ecological genomics (Narum *et al.* 2013).

II. Candidates for Domestication

Perennial sunflowers (*Helianthus* ssp., Asteraceae)

Annual sunflower (*Helianthus annuus* L.) is the only major grain crop domesticated in North America. The domestication of annual sunflower is believed to have been rapid as most of the traits which distinguish wild and domesticated plants such as achene and disc size are polymorphic or quantitative traits that show continuous distributions (Burke *et al.* 2002; Chapman *et al.* 2008; Blackman *et al.* 2011; Chapman and Burke, 2012).

Similar to annual sunflower (*Helianthus annuus* L.), the perennial North American species Maximilian sunflower (*Helianthus maximiliani* Schard.) and stiff sunflower (*Helianthus pauciflorus* Nutt.) have a range that encompasses most of North America, stretching from southern Texas to southern Manitoba. Both *H. maximiliani* and *H. pauciflorus* die to the ground each autumn surviving the winter through tuberous rhizomes. Similar to other wild sunflower species (including wild *H. annuus*) they are self-incompatible outcrossers and the seed requires an extended (4-6 weeks) cool, moist stratification period for full germination.

H. maximiliani and wild *H. annuus* show clinal variation for a number of morphological and life history traits influenced by genetic drift and natural selection (Blackman, Michaels, and Rieseberg 2011; Kawakami *et al.* 2011). For example, Kawakami *et al.* (2011) found that Maximilian sunflower traits such as flowering time, disc size, plant height and growth rate exhibit a clinal distribution across a latitudinal gradient of North America. Discs ranged between 9.8-26.2 mm in populations collected from the southern range of the species in Texas to its Northern range in Manitoba.

The range of habitats in which these widely distributed perennial species persist leads us to predict that, like the wild annual sunflower, they harbour the genetic variation necessary for domestication. New mutations may not be required. Consistent with this prediction, we have found a great deal of variation for traits of interest within and between accessions collected from our locations in Kansas and Manitoba. Families collected across Manitoba grown under growth chamber conditions show considerable variation in plant height, days to flowering, disc size (3.8-18.3 mm), and branching architecture.

Helianthus maximiliani: Maximilian sunflower grows vigorously as a seedling, flowering in the first year. It has good seed fertility and well documented disease resistance including rust and *sclerotinia* (Rashid and Seiler, 2004; Muellenborn *et al.* 2011) and seed oil content equal or surpassing that of wild annual sunflower (Seiler and Brothers, 1999). The oil is rich in the essential fatty acid linolenic acid and higher in tocopherols (compounds with vitamin E activity) than any other wild Helianthus species evaluated (Velasco *et al.* 2004). As with many species of Helianthus interspecific gene transfer is possible though the viability and fertility of F1 interspecific hybrids is low (Atlagic *et al.* 1995). Chromosome doubling through colchicine has been proven to be effective in developing fertile *H. annuus* X *H. maximiliani* amphidiploids (Feng and Jan, 2008) and *H. maximiliani* has served as a source of novel restorer genes and male-sterile cytoplasms in *H. annuus* for hybrid breeding.

Maximilian sunflower is being domesticated simultaneously in Kansas and Manitoba. While genetic materials have been exchanged between these programs, the day length and climate differences make it probable that two distinct domesticated races are likely to emerge. In both locations the basic approach has been to collect seeds from a number of wild populations in the region of the breeding station, stratify seeds and transplant the seedlings to the field for evaluation. The program in Manitoba is still in the evaluation phase, although crosses have been made between wild Manitoba and improved Kansas individuals for the purpose of developing mapping populations. Initial results suggest that wild Manitoba populations are capable of persisting and will readily set seed in nutient deficient soils. Phophorus and nitrogen levels ranging between 2-16 ppm and 4-36 lbs/acre. respectively are capable of sustaining large stands though the total number of seed bearing heads per plant are reduced under these conditions. Substantial variation in floral disk dimaeter, plant height and days to flowering have been observed between sub-populations in Manitoba suggesting that there is enough genetic variance to make genetic advancement for these traits within the population base.

In Kansas, selections from the initial evaluation nursery were intermated in 2003 to produce Cycle 1 plants. Selections from Cycles 1, 2 and 3 were intermated in 2006, 2009 and 20012 respectively. The general method has been to plant spaced-plant evaluation nurseries, select 20-50 individual plants using information about the performance of each plant's half siblings in the second year, dig the selected plants early in the third year and polycross them in a crossing nursery. For polycrossing, controlled pollinations were made using a bulk of pollen obtained by pooling pollen from the selected plants. Although many traits have been measured over the years, the selection indices were based primarily on seed per stalk, seed per head and average seed mass.

A side-by-side, multiyear comparison of the yield of selected Cycle 3 plants, random unselected Cycle 3 plants, and wild plants is currently in the field, so we cannot definitively estimate the gains made to date. However, heritabilities of the traits under selection (Table 1) and the data from each population (Figure 1), though grown in different years, suggest that yield has increased.

FIGURE 1. AVERAGE YIELD COMPONENTS OVER SEVERAL YEARS IN A *HELIANTHUS MAXIMILIANI* POPULATION UNDERGOING ARTIFICIAL SELECTION FOR INCREASED SEED YIELD

Each point on the graph shows traits from plants sampled in their second year (after transplanting). Experimental designs are summarized in Table 2. All experiments were established in Salina Kansas by transplanting clones or seedlings and were managed without fertilizer addition or irrigation beyond what was required to establish transplants. Weeds were controlled by cultivation except in the case of Experiment 2 (2005) where sunflowers were intercropped with alfalfa (*Medicago sativa*). The alfalfa and weeds were controlled by mowing between rows and within rows as necessary.

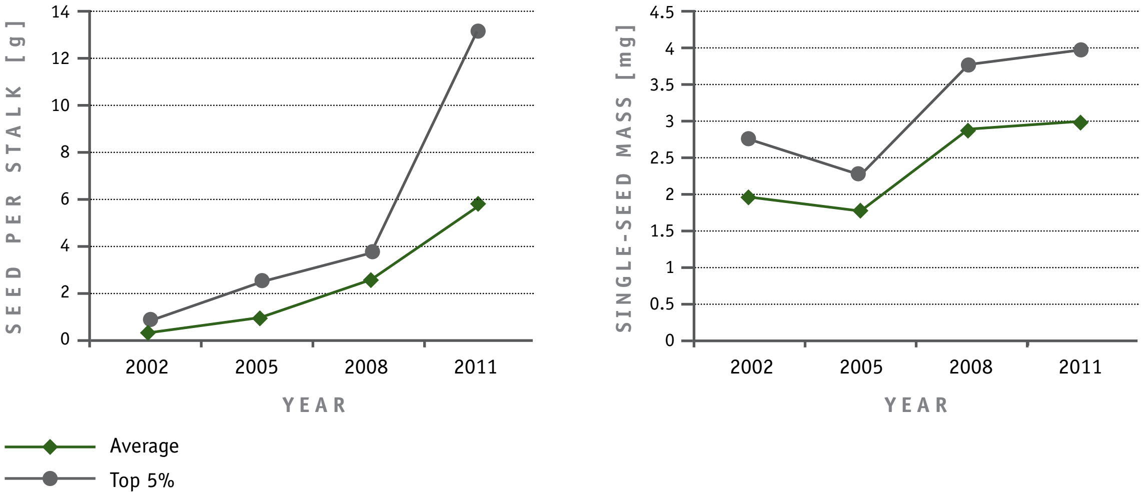

TABLE 1. MEANS, STANDARD DEVIATIONS AND RANGES, AND HERITABILITIES FOR SEVERAL TRAITS MEASURED IN SEVERAL DIFFERENT BREEDING POPULATIONS

Experiments were performed in different years and traits were not always measured the same ways, so only approximate comparisons are possible. Seedlings were germinated in controlled conditions and transplanted to the field in May. All experiments were performed in Salina, Kansas without fertilizer application or irrigation beyond the minimum required for transplant establishment. Only data from 2-year old plants is shown here unless otherwise indicated. Heritabilities were calculated from individual genotypes in known half-sib families using REML processes (Genstat, London) that included spatial components and blocking terms in the model only if they improved the model for a particular trait.

SPECIES, YEAR	NUM. PLANTS PHENOTYPED [NO. HALF-SIB FAMILIES]	SPACE BETWEEN ROWS (AND WITHIN ROWS) (m)	SINGLE-SEED WEIGHT (mg)			SEED YIELD PER STALK (g)		
			Her	Mean (stdev)	Range	Her	Mean (stdev)	Range
S. integrifolium 2008	2246 [224]	0.9 (0.9)	27	23.7(3.9)	12.0 - 42.5		10.9 (9.9)	0 - 83.1
S. integrifolium 2012	2720 [109]	0.9 (0.45)	61	15.6 (3.3)	5.6 - 29.5	27	3.46 (2.3)	0 - 19.9
H. pauciflorus 2012	2555 [100]	0.9 (0.45)	69	8.2 (1.7)	2.0 - 14.9			
H. maximiliani 2008	2240 [20]	0.9 (0.9)	27	2.86 (0.05)	0.42 - 7.68	14	1.33 (1.07)	0.01 - 12.9
H. maximiliani 2011	710 [50]	1.8 (1.8)	34	2.98 (0.80)	0.407 - 15.7	42	5.74 (3.72)	0.01 - 36.9
H. maximiliani 2012*	1925 [113]	0.9 (0.9)	49	4.88 (0.75)	3.33 - 7.65			

* Data taken in first season on a subset of plants, selected visually for reduced branching

SEED YIELD PER HEAD (mg)			SEED YIELD PER HEAD AFTER SHATTERING (mg)			PERCENT NOT SHATTERED			OTHER TRAIT OR NOTES			
Her	Mean (stdev)	Range	Her	Mean (stdev)	Range	Her	Mean (stdev)	Range	Trait	Her	Mean (stdev)	Range
13	423 (142)	0 - 992							Stalk diameter		9.78 (3.6)	2 - 20
									Num ray florets per head	78	51.8 (13.6)	8 - 165
71	291 (197)	0 - 135	67	107 (82.2)	0 - 641	42	43.3 (22.0)	0 - 100	Seed not shattered (%)	42	43.3 (22.0)	0 - 100
42	415 (122)	14 - 844		35.0 (27.1)	0 - 824	16	10.74 (7.46)	0 - 100	Number of stalks per plant	16	19.4 (8.97)	2 - 80
	206 (112)	3.17 - 554		12.2 (13.1)	0 - 153.5	51	9.56 (12.9)	0 - 96.8	Number of stalks per plant	48	24.3(11.0)	3 - 75
31	640 (362)	0 - 2240	33	296 (199)	0 - 1470		48.9 (20.3	0 - 100				

In 2007, a single unusual individual was observed in the Cycle 2 evaluation nursery. This plant had strongly reduced branching and a single, larger than average head on each main branch. Open pollinated progeny from this plant were intermated in 2008 to produce a segregating population in 2009. Plants with strongly reduced branching in their first season were intermated that same year and this process was repeated in 2010 and 2011. Data from the initial reduced branching segregants (2009) show reduced seed mass per head thereby confirming our observation of malformed heads and florets (Figure 2). By 2011 the average yield per head was approximately equal to that of the population from which the first reduced branching plant was taken in 2007, and some individuals had yields per head exceeding anything in the normally branching population, again, confirming our observation that reduced branching is associated with a wider range of disk diameters. We attribute the restored seed fertility to natural selection in which more fertile individuals contributed more pollen and more seeds to the subsequent generation than less fertile individuals. Reduced branching materials are currently being crossed with wild-type materials from Manitoba to develop mapping populations and to introduce this trait into a northern adapted genetic background to expand the diversity of the uniheaded lines and develop divergent breeding pools.

FIGURE 2. QUANTITY OF SEED PER HEAD HISTOGRAMS SHOW CHANGES IN A *HELIANTHUS MAXIMILIANI* POPULATION UNDERGOING ARTIFICIAL SELECTION FOR INCREASED APICAL DOMINANCE

Heads were sampled in the same year as seedling establishment. The 2007 sub population is the same as the population shown in Figure 1. Open pollinated progeny of a single unusually large-headed, nearly unbranched plant in that experiment were intermated to produce the sub-population evaluated in 2009.

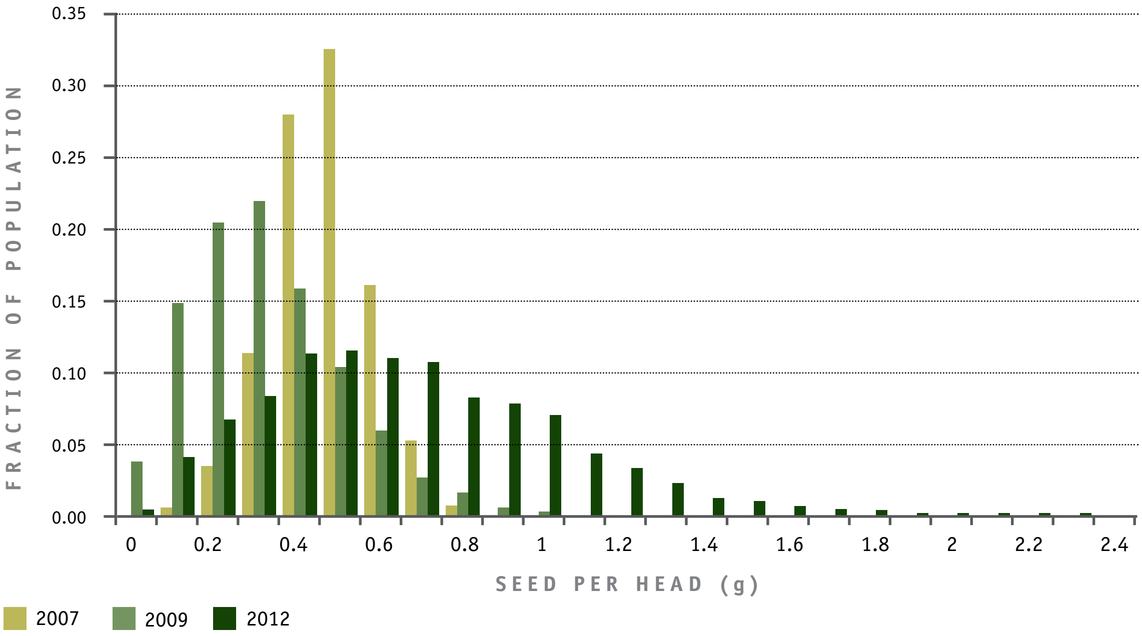

TABLE 2. EXPERIMENTAL DESIGNS

EXPERIMENT	YEAR PLANTED	YEAR DATA TAKEN	EXPERIMENTAL FACTORS			SELECTION		NOTES
			Plots	Number of families	Number of genotypes	Num. of genotypes selected to advance	Percent of genotypes selected to advance	
1	2001	2002	960	96	960	150	15.6	Families are batches of seeds collected from different wild populations
2	2004	2005	900	15	150	20	13.3	Genotypes in this experiment are a subset of the genotypes in the first experiment. Selected individuals were dug and their rhizomes divided and transplanted to the new nursery with clonal replication
3	2007	2008	2240	20	2240	50	2.2	Selected plants from Experiment 2 were intermated to produce seed for Experiment 3
4	2010	2011	710	50	710	50	7.0	Selected plants from Experiment 3 were intermated to produce seed for Experiment 4

Helianthus pauciflorus* (= *H. rigidus*) and *H. tuberosus: these are tuberous, hexaploid perennial sunflower species (n = 51). We consider them together here because they are known to hybridize naturally forming fertile populations (Brooks and Schofield, 1986) that have previously been given species status and are now considered subspecies or hybrids. Clevenger and Heiser (1963) commented that "In their extreme forms, the [hexaploid *Helianthus*] taxa considered here are quite distinct, but intergradations are frequent in nature." In Kansas, *H. pauciflorus* and *H. tuberosus* are readily distinguished when collecting seeds or plants from wild populations. However, open-pollinated progeny of wild-collected individuals grown in a common garden are much more difficult to categorize than their parents, presumably due to interpollination.

Both tuberous species were used as parents in interspecific crosses with *H. annuus,* mainly between 2004 and 2009. *H. pauciflorus* gained attention as a candidate for direct domestication because some individuals had larger seeds than *H. maximiliani* and large heads bourn on long peduncles. The branching pattern and peduncle length place the infructescences largely above the leafy portion of the plant, making mechanical harvesting easier. Although The Land Institue population under consideration for perennial oilseed development is referred to as *H. pauciflorus* it could more accurately be considered a hybrid swarm with individuals positioned all along the intergradation between the two wild hexaploid species.

Both tuberous species are sources of resistance to rust (*Puccinia helianthi*) (Zimmer and Rehder 1976), and both are crossable with *H. annuus*, though, as described above, the hybrids are only weakly fertile (Georgieva-Todorova, 1964; Jan, 1997). Despite the barriers to gene flow, *H. tuberosus* has been the source of important disease resistance genes in *H. annuus* (Rogers *et al.* 1982), and *H. pauciflorus* has provided cytoplasmic male sterility genes (Jan *et al.* 2002). The cytoplasm of many commercial sunflower cultivars may actually be from *H. tuberosus* (Jan and Ruso, 2000). *H. tuberosus* was domesticated as a tuber crop and both tubers and vegetation could be used for bioethanol production or livestock fodder (Kays and Nottingham, 2007). *H. pauciflorus*, though never domesticated, is also strongly tuberous.

The yield potential of this species remains unknown, though initial field trials were established at the Land Institute in 2011, the University of Manitoba in 2012, and Organic Valley Co-op (Cashton, Wisconsin) in 2013 to evaluate this species. Perhaps because of the large phenotypic variation resulting from the intergradation between the two species and their respective hexaploid genomes, the heritabilities for traits of interest measured in a large Land Institute evaluation nursery in 2012 were high (Table 1), suggesting that a breeding program could be successful. However, the extreme sensitivity of sexual reproduction in this population to drought led the Land Institute to phase out its evaluation of this species in Kansas, focusing efforts on the collaborative breeding effort in Wisconsin where this species may be better adapted to the shorter growing season and lower potential evapotranspiration.

Helianthus cusickii: Cusick's sunflower is a diploid perennial (2*n*=34) adapted to dry, rocky soils of high mountain desert, surviving with annual precipitation of only 25-60 cm (Seiler, 1992) by means of a robust taproot. It flowers earlier in the year than the other perennial sunflowers discussed here and is noted for morphologically being the most extreme species in the genus (Heiser, 1965). Counterintuitively, it is more closely related to *H. annuus* than the other perennial species that have been used as sources of genes for the improvement or perennialization of *H. annuus* (Timme *et al. 2007)*. However, both pre and post zygotic barriers to gene flow are expected (Sideli *et al.* 2013). We have made preliminary attempts to produce interspecific hybrids between *H. cusickii* and *H. annuus*. Putative hybrids are being observed and additional attempts at hybridization will be made and it remains to be seen if genes for perenniality, fleshy taproots, or other drought tolerance traits can be introgressed to the annual crop sunflower.

Its early flowering, small pubescent leaves, modest height and deep roots make this species a possible candidate for domestication as a drought tolerant oilseed, especially if domestication related genes could be introgressed from *H. annuus*. However, after planting out hundreds of individuals representing a large portion of the species' native range (seeds collected in California, Nevada, Washington and Oregon) in typical agricultural soils in Kansas, we found poor survival and low vigor compared to several other annual and perennial *Helianthus* species. Possibly this species has not been exposed to soil pathogens typical to agricultural soils but uncommon in desert soils. However, a few individuals did survive into a second year and, if intermated, could be used to develop a population with greater adaptation to agricultural conditions.

CANDIDATES FROM OTHER GENERA

While the genus Helianthus is an obvious source of perennials that could be domesticated as oilseeds because it includes numerous perennial species as well as two domesticated species (*Helianthus annuus* and *Helianthus tuberosus*), the North American grasslands contain many other species with oil rich seeds. Some of these are likely to have chemical or anatomical adaptations not available in *Helianthus*.

***Silphium* (Asteraceae):** The species of the genus *Silphium* have much larger seeds (e.g. 21 mg reported by Kowalski and Wiercinski, 2004) than most other native North American forbs and grasses. The flavor and composition of major nutrients is similar to that of the domesticated sunflower (Kowalski and Wiercinski, 2004). The heads resemble that of a sunflower, but whereas the disk florets of *Helianthus* are perfect and the ray florets are sterile, in *Silphium* the ray florets are pistilate and the disk florets are staminate (Brooks and Schofield, 1986). Several observations of wild stands of *Silphium integrifolium*, a fairly common prairie species in Kansas, led us to consider this species for domestication. In addition to the size of its seeds, we observed variable seed shattering. Mature seeds remain on some heads well into the autumn or even the winter. This is easy to see because the large seeds remain attached to the heads after other floral structures and sterile florets have fallen off. The other key observation was the performance of this species during dry intervals or even serious droughts. In 2012, the drought in Kansas was severe enough that test plots of commercial hybrid sunflowers administered by Kansas State University in nearby Hutchinson, Kansas, were terminated due to crop failure (Stewart Duncan, personal communication). The average seed yield of S. integrifiolium in The Land Institute breeding nursery, where plants had been transplanted the year before in 0.9 m rows, 0.45 m within rows, with no fertilization, was 312 kg/ha and some exceptional plant were estimated to have yielded up to 1182 kg/ha.

In 2001-2002 we evaluated both *Silphium integrifolium* and *S. laciniatum*, another drought tolerant, perennial species, for their adaptation to agricultural conditions. We found that neither species flowers in the first year. They produce large basal leaves and no stems. In the second year *S. integrifolium* reliably produces stout stalks and flowers. *S. laciniatum* is much slower to develop and does not always flower in the second year. We judged its establishment phase to be too slow for agriculture and focused on *S. integrifolium*.

The most obvious barrier to high seed yield in all *Silphium* species is the production of seeds by only the ray florets which number from 15-32 per head in *Silphium integrifolium*. To test whether genetic variation existed for this trait, and whether the number of fertile florets could be increased to at least 100 per head, we practiced recurrent selection for that trait alone. Seed from 21 Kansas populations were allowed to interpollinate in 2004. Approximately 100 000 progeny were planted in 2005 and screened in 2006. Because scoring the number of ray florets is very time consuming, we never measured the population average thoroughly, but estimated it to be about 28-30. The ray florets on plants visually identified as having heads with high numbers of ligules (the long, strap-like corollas of ray florets) were carefully counted. Eighty three plants with 38 or more ligules were found and intermated that year. The progeny of this polycross were planted in 2007 and similar recurrent selection and intermating was done in 2008 and the process repeated in 2009-2010. In 2012, finding that the average number of ray florets in a population of 2 720 was 52, that several plants had over 100 ligules and that one had over 150, instead of simply intermating the plants with the most ray florets, we decided to make selections using an index based on seed yield per stalk and seed size (mass). We reasoned that we had good evidence that the population contained adequate genetic variation for ray floret number and that pushing the population mean higher prematurely might result in loss of variation for seed size or other traits of interest. Furthermore, some of the plants with very high ray floret numbers are now close to being male sterile.

***Linum* (Linaceae):** Lewis flax (*Linum lewisii* Pursh.) and stiffstem flax (*Linum rigidum* Pursh.) are both perennial North American species in the same genus as domesticated annual flax (*Linum usitatissimum*). Antinutritional saponins are reported in *L. rigidum*, limiting its usefulness to agriculture. Cultivars of *L. lewisii* with improved longevity, drought resistance and disease resistance have been developed as ornamentals and for use in landscape reclamation. This species also has good forage value.

L. lewisii has fewer chromosomes (n=9) than annual domesticated flax *Linum usitatissimum* L. (n=15) which could pose a barrier to interspecific hybridization. Fortunately unlike the large complex genome of polyploid candidate perennial grain species (perennial wheat ~17 Gb) or those with highly repetitive genomes (*Helianthus* spp. ~3.5 Gb) the flax genome is small (~370 Mb) and whole genome resequencing is emerging as a technique for improving domesticated flax (Fu, 2012). The small genome size in the genus *Linum* in theory should facilitate whole genome association studies into the genetics of domestication and for screening germplasm for favorable traits.

III. Discussion

Assuming that we have successfully argued the case for domesticating perennial oilseeds, we (authors, scientific community and society) are still faced with difficult decisions about which candidate species are most promising.

The candidates from the Asteraceae, described above, provide an excellent study in contrasts between "perennials." Whenever we have done the experiment, we have found that the traits of interest are heritable. It is difficult—maybe impossible—to judge which species has the highest yield potential, given unlimited cycles of selection for yield. We argued above that it might be simpler to domesticate species by directional selection on quantitative traits like yield and (in sunflowers) shattering than to attempt to introgress complex traits such as lifespan. The same logic may apply within the subset of perennial species. Root structure or vegetative spread by tubers may involve complex developmental pathways. Other traits may simply be very expensive to measure due to the instrumentation required or need for controlled/multiple environments. It would be well to consider the following traits carefully when selecting a perennial species for further investment.

ABOVE-GROUND PLASTICITY

To our surprise, we discovered that *H. pauciflorus* and *H. maximiliani* plants when dug from a mature prairie and transplanted to an experimental plot were likely to triple or quadruple in height. Leaf size, stem diameter and number of inflorescences are also altered in this environment. In short, the plants look like a different species. One implication of this phenomenon is that it is impossible to judge the agricultural phenotype of a wild species without growing it in rows or as spaced plants in an experimental plot where they have a different nutrient status and are released from competition with other plants. A more subtle implication is that we have found that plants with this level of plasticity are highly sensitive to both drought and self-crowding and may require nutrient limitations to modulate overabundance of growth. A positive implication may be that these species are capable of producing seed under resource poor conditions.

Domesticated sunflower has a high rate of evapotranspiration, a trait often exploited as an ecosystem service to lower the water table in areas dealing with salinity. Under drier conditions this can result in drought stress later in the growing season greatly impacting yield potential because the reproductive growth stages of sunflower are more sensitive to moisture stress than the vegetative stages (Göksoy *et al.* 2004). In the southern part of its range (e.g. Kansas), Maximilian and stiff sunflower undergo an extended vegetative period beginning with emergence in May, expend soil moisture and accumulate biomass throughout the season. If moisture or fertility is available early in the season, plants can grow quite tall and branch freely. Frequently as temperatures rise throughout the season these species often endure mid-season drought resulting in a loss of accumulated biomass and potential volatization of nutrients prior to reaching its necessary short day photoperiod requirement for flowering in September.

Thus the plasticity of *H. maximiliani* and *H. pauciflorus* cause—from the perspective of maximizing seed production per unit area—excessive vegetative growth early in the season and excessive vegetative growth in high rainfall years. Both problems result in reduced harvest index (if abscised leaves are included in the calculation) and premature depletion of soil moisture and potential for nutrient volatization. A second issue is that many of the phenotypes of interest to breeders are likely to respond to the environment in ways that are difficult to quantify, impeding breeding progress. For example, small scale patchiness in soil texture or drainage within a breeding nursery can be difficult to model using conventional blocking or even spatial statistics. Neighboring plants also modify each other's environments in ways that are difficult to control or model especially early in the domestication process when the phenotypic variance of the population is great, when gaps are produced irregularly in the field due to plant mortality, or when transplanting or cultivating errors create slight differences in the spacing between or within rows.

In contrast, *H. cusickii* and *S. laciniatum* showed only modest, if any, increase in growth or stature when grown with reduced competition. Unfortunately, this makes them very uncompetitive with weeds, at least in their first few years, and therefore difficult to work with. *S. integrifolium* has an intermediate response growing about twice as tall in cultivation than in the wild. This species also appears to have much greater seed yield and height stability from year to year. One reason for its reduced plasticity is that like the other species it ceases to grow in height at the time of flowering but unlike the Helianthus species, in Kansas it flowers beginning in early July whereas *Helianthus pauciflorus* begins flowering in late August and *H. maximiliani* in early September.

This gives us a clue about a strategy for reducing the plasticity of the *Helianthus* species in the southern part of its range: introgress flowering time genes from points further north. Manitoba *H. maximiliani* populations flower in July.

RHIZOMATOUS SPREADING

S. integrifolium is less plastic than *H. maximiliani* and *H. pauciflorus* in another way: it forms a crown that does not increase in diameter much over time. This means that the number of stems per unit area increases very slowly, if at all (after the establishment year). At the other extreme, *H. pauciflorus* produces rhizomes that radiate 30-50 cm from each stem in all directions. The number of stems per unit area increases geometrically from year to year, leading to severe crowding and stunting of the stems. Without thinning, by the third season, the plants are as short and produce as few heads as when this species is found growing in a mature prairie.

Aggressive asexual spreading impedes plant breeding progress because adjacent genotypes in a nursery are usually impossible to separate in the second season when they are regrowing from large overlapping rings of rhizomes/tubers. This makes it difficult and risky to make selections of individual plants based on their reproductive traits from the year before. Instead, a subset of individuals in a segregating population would be visually selected and their tubers dug before the rhizomes connecting them to the original seed-grown plant rot. Tubers would be planted in small plots and genotypes selected for intermating on the basis of plot yield. Although this digging of tubers and establishment of plots of clones would be labourious and eliminate the possibility of making selections the first year after intermating, some traits could be measured this way that cannot be measured on individual spaced plants (see the Tubers section below).

A more serious problem is the phenomenon of stand decline. *H. pauciflorus* stands decline in seed yield rapidly after the first year. As described above, as stalk density becomes greater, the stalks become shorter and more slender. Many completely fail to flower and those that flower produce much smaller heads. We have not studied the effect of ramet density on root traits, but we hypothesize that roots also become shorter and more slender. The "luxury uptake" of water by such dense stands early in the season may be another factor in stand decline. A second-year population in Kansas, 2012—a dry year, declined so strongly that by midsummer most stalks were extremely stunted and had only brown leaves. In contrast, *S. integrifolium*, planted in the same field at the same time grew and flowered normally.

TUBERS

The tubers of *H. pauciflorus* and *H. tuberosus* are a curse when trying to achieve and maintain an optimal plant density, as described above, but also a potential blessing. Although asexual reproduction of this kind would require farmers to diligently cultivate between and possibly within rows to maintain stalk density within target range, it would also provide an easy method for propagating genetically uniform cultivars, bypassing the need for years of inbred line or synthetic cultivar development. The tubers are easy to dig, store and transport, fairly uniform in size, and could be planted with standard potato planting machinery. Possible applications of tuber propagation:

1. Fixed specific combining ability: Fortuitous crosses creating even a single outstanding F1 individual could be all that is required to instantly create a superior genetically fixed cultivar despite chromosome compliment. Emasculations and careful pollination would not be required to generate propagules each year. And unlike potato, where micro-tubers are sometimes produced in vitro to reduce the costs of shipping whole field-grown tubers or cutting them into pieces, the tuberus rhizomes of *H. pauciflorus* and *H. maximiliani* are already small.
2. Locally adapted cultivars: Ecovars (TM)—improved ecotypes—have been proposed as a way of exploiting the adaptations that have evolved in local populations of a species. Attempting to produce numerous locally adapted cultivars of an outcrossing species, while retaining hard-won domestication traits and population uniformity in phenology and plant height, is a daunting prospect. Paradoxically, the possibility of eliminating genetic variation at the field

scale through asexual propagation may make it more feasible to release numerous genetically distinct locally adapted cultivars.

3. Ecological genomics research: Ecological genomics aims to 'find the genes that matter', that is those who influence adaptive variation in the context of their natural environments (Edwards 2013). Being able to propagate individual genotypes asexually (and inexpensively) would allow new phenotypes to be mapped in segregating populations or diverse association mapping panels. The phenotypes of most interest to agronomists, yield, water use, nutrient uptake, stalk density over time, are so easily confounded by inter-genotype competition and plant spacing, that they can not be meaningfully measured on individual seedling-grown plants. These traits can be measured using inbred families derived from segregating populations, but this is much more difficult to accomplish with outcrossing species and likely to be confounded by inbreeding depression therefore association as opposed to pedigree based approaches may be better suited for these species.

ROOTS

Belowground traits are difficult to observe and, therefore, difficult and expensive to change directly using conventional phenotypic selection. The range of root traits among seed-producing perennials is very great. In selecting between candidates for domestication as new crops, it would be wise to carefully consider which kind of root system is needed to provide the desired ecosystem and economic services in the target environment. We have speculated that with a few exceptions (wheat, sorghum) it may often prove to be easier to domesticate species in order to capture their life history and cold tolerance traits (perenniation) than to try to breed or engineer such complex traits in an annual crop using de novo variation or variation brought in from wild relatives. If this logic applies to the easily scored trait of winter survival, it is applies more strongly for the possibly equally complex rooting traits which are far more expensive to "phenotype" under realistic field conditions.

In contrasting the response of experimental populations of *Silphium integrifolium*, *Helianthus maximiliani* and *H. pauciflorus* to drought it is impossible to separate the effects of root depth and increasing stem density. Adjacent, same-age plots of *S. integrifolium* appeared unwilted throughout the 2012 growing season while in midsummer, *H. pauciflorus* appeared scorched and green material was only seen after autumn rain and lower temperatures. The response of *H. maximiliani* was intermediate. The plants were shorter than normal, many leaves dropped, and the remaining leaves were permanently wilted during July and August. A counterintuitive aspect of these observations is that *H. pauciflorus* is often found in dry, rocky, shallow-soil locations, while *S. integrifolium* is often seen at lower landscape positions where soil and moisture are more abundant.

Weaver and Stoddart (1935) noted similar contrasts between *H. pauciflorus* and *S. integrifolium* during the drought of 1934 (the "dust bowl" years), writing, "A few species such as … *Silphium integrifolium*, grew normally and stood unwilted above the dried grasses." They attributed the drought avoidance of these forbs to their deep roots: "Among the forbs, resistance to drought was closely correlated with root extent. Species with root systems penetrating 8 to 20 feet into the moist subsoil were little affected."

These authors also noted that, "*Helianthus rigidus* [= *H. pauciflorus*] was often completely wilted and the leaves dried [in the prairie] while a foot or two distant in cultivated land the same species was 3 feet or more tall, abundantly supplied with flower heads and completely turgid." This statement is consistent with our observations of great differences in height, number of heads and drought stress between seedlings ("cultivated land") and second or third year stands in which the plant density approaches that of the prairie and presumably results in soil water depletion. The authors clearly characterize this species as drought tolerant, stating that "the tenacity with which [*Helianthus rigidus*] persisted after weeks of semi-wilting was impressive."

In climates experiencing variable rainfall within and between growing seasons, the advantages of drought avoidance through persistent deep root systems over mere drought survival through osmotic adjustment, wilting, and stunting seem obvious. For subsistence agriculture, yield stability is more important than yield potential, and crops that yield well during dry years would increase food security.

Increasing the rooting depth of crops has been proposed as a method for sequestering meaningful amounts of carbon (Kell, 2011). If deep roots persist from one year to the next (low turnover) they may not require much more photosynthate for maintenance than shorter root systems. However, in the first few weeks or months of life, the construction of this root system will almost certainly require greater allocation of photosynthate belowground than for other types of root systems. The cost, then, is likely to be reduced aboveground growth in the first year. This translates to poor weed competition (*Silphium* spp. and *H. cusickii*) and/or no flowering in in the first year (*Silphium* spp.). A less obvious side effect of deep persistent roots is reduced seedling nutrient and water acquisition. The surface area of a few large-diameter seedling roots heading almost vertically downwards must be lower than a finer more branching seedling root system. We have seen that following transplanting *S. integrifolium* seedlings are more vulnerable to water stress, experiencing higher mortality in unirrigated plots, than perennial *Helianthus* seedlings. Thus long-term drought avoidance comes at the cost of poor drought tolerance, especially in the first few weeks.

We do not know the costs or benefits of the various rooting strategies in other circumstances. In locations with poor drainage or frequent floods, are fast-growing "disposable" root systems a better investment than costly, deep, permanent ones? Which root systems tolerate root predation better? Do root systems with large root systems and low root turnover tend to immobilize soil nitrogen leading to nitrogen deficiencies?

APICAL DOMINANCE

Dramatic changes in aboveground branching have happened during the domestication of many crops including annual maize and sunflower. So on the one hand, the branching pattern of a wild plant need not eliminate it from consideration for domestication. On the other hand, branching in perennials is much more complex than in annuals. Herbaceous perennials regulate their architecture by controlling branching and internode length at several points including the below-ground branches (rhizomes, tillers), main lateral branches on stalks, and short flowering branches.

As described above, *Helianthus maximiliani* families with a single head per stalk are now available, as are multiheaded types and intermediate lines that produce only a few short flowering branches just below the terminal inflorescence. Increased apical dominance resulting in fewer, larger infructescences and resulted in the characteristic large head of domesticated sunflower. Depending on the context of the habitat in question apical dominance is observed to both positively and negatively impact relative fitness (in the form of intact harvestable seed) in annual sunflower (Reagon and Snow, 2005). The development of a single determinant head increases seed size but can leave the plant vulnerable to seed predation or head loss by the native head clipping weevil. Reduced branching and number of sub-terminal inflorescences on the main stem increases both the synchrony of head maturation and the uniformity of head position on the plant. Both facilitate harvest and reduction in losses to seed predators and shattering. Synchrony of anthesis and maturation means that mature heads do not need to be left in the field—where they are exposed to shattering and bird predation--while immature heads continue to develop. Positioning heads near the top of the plant means that less leafy material must be processed by mechanical harvesters. Excessive foliage near the heads can slow the harvesting process, require suboptimal winnowing settings, or result in contamination of the harvested seed by wet material.

In general, apical dominance combined with early flowering may present an opportunity for increasing the harvest index of perennial oilseeds while reducing the phenotypic plasticity. The longer growing season of perennials compared with annuals allows them to produce more biomass, but in opportunistic, highly indeterminate species, even if stems were harvested as a co-product with the seeds, much of this productivity may be unharvestable (dropped leaves, withered, headless stems, aborted heads) in a dry year or present harvesting difficulties (thick woody stalks, excessive wet, green material) in a good year. Where oil production is the main goal it is clear that either the plants must stop growing vegetatively and enter the sexual phase early in the growing season or that the plants must be low-growing early in the season, accumulating carbohydrate reserves belowground and then bolt late in the season, producing heads on largely leafless stems.

While in theory breeding *H. maximiliani* to flower earlier in the season in the southern part of its range could accomplish these goals, it remains to be seen whether this will succeed. One possibility is that after flowering, apical dominance will be released and new primary stalks may emerge from the rhizomes or the existing stalks may continue to branch and produce leaves without a means of accumulating reserves for the following year. Another possibility is that the plant may simply die back. This would accomplish the goal of conserving soil moisture for the next season, but would reduce the productivity of the cropping system in good and average years. Both of these phenomenons have been observed under growth chamber conditions suggesting possible genetic variance for the regulation of rhizome dormancy following flowering and could potentially be manipulated as well. In the case of tuberous perennials, early flowering may help reduce the harvest index, but the opportunity for storing carbohydrates after seed filling is limited. This is because the main sink at this point is the new tubers, but only a single replacement tuber is required to maintain the stand density and excess tubers may need to be removed to prevent overcrowding. For regions with short growing seasons, these points may be irrelevant. Water stress may be a rare problem and winter imposes a limit to excessive vegetative growth. This may be the only suitable environment for tuberous perennials.

SENESCENCE

One of the most useful features of the annual habit is the programmed death of the stems and leaves bearing the heads to be harvested. Senescence allows the grain and the stem to dry prior to harvest. While the individual heads of the native oilseeds surveyed here senesce when the seeds are mature, the stalks and leaves generally do not until killed by frost. At that point, the weather is cool and the plant material dries slowly. Opportunities for remobilizing biochemical contained in the leaves and stems are also missed. Conversely under growth chamber conditions following flowering senescence has been observed in plants despite optimal temperature, moisture and nutrient conditions suggesting a killing frost may not be necessary to trigger senescence.

If candidates with autumn foliar senescence are not found, chemical defoliants--already used to facilitate dry down and harvest of some annual grain and perennial hay crops-- could be used with perennial oil seeds. An alternative would be to look for species that produce most of their leaves close to the ground and send up a specialized stem with fewer or smaller leaves to display the inflorescences above the canopy. Even if this stem did not senesce after seed maturation, it might be less succulent and leafy than the stems of other species and thus present fewer problems for harvest. Some prairie plants produce such structures, including *Tripsacum dactyloides*, *Silphium laciniatum* and *S. terebinthaceum*. Perhaps these *Silphium* species should be re-evaluated as potential oilseeds. It would be necessary to demonstrate that such a species also had genetic variation for increasing the diameter and rigidity of such a stem in order to bear the weight of larger, heavier seed heads.

Plant senescence in annual crops also gives the farmer a high level of control over the plant density each year. We alluded above to the potential problem of ramet proliferation through rhizomatous and tuberous asexual propagation. Even when the fine roots or leaves of perennials senesce, shoot and root apical meristems may not. With woody crops, pruning to control the number of aboveground meristems (buds) is crucial for maintaining fruit size and plant productivity from year to year (Marcel-les-Valence, 2006). In the absence of programmed meristem population control, agronomists may need to develop methods to “prune” back the above and below-ground meristems of perennial herbs.

The predictable senescence of all the plants in a field of an annual crop provides an opportunity for tillage or pesticide application to kill any invading vegetation and to disrupt the lifecycles and habitats of many kinds of agricultural pests. Of course, the downside of this is that the soil is exposed to erosion and the habitat of beneficial or threatened animal species is compromised. How can perennial grain farmers achieve the necessary function resetting secondary ecological succession annually without the options available to annual grain farmers? Controlled burning and grazing have been used for centuries to arrest succession at a point that provides abundant palatable herbaceous species for grazing livestock. Both of these interventions can prevent the invasion of woody plants that lose their competitive advantage when their aboveground meristems are killed each year. However, many kinds of perennial or biennial weeds are herbs and tolerate these stresses necessitating a diversified integrated approach. We have found that improved populations of perennial oilseed candidates, once established are quite competitive with other herbs and prevent their invasion. Breeding to increase determinacy, advance flowering in the season, reduce asexual reproduction, and increase harvest index is likely to make these species less competitive with other herbs. Perhaps this is an ecological tradeoff. An agroecosystem in an un-invadable state is also in a state in which asexual structures and processes dominate. The *H. pauciflorus* population at The Land Institute in 2013 illustrates this. The density of ramets has increased each year since seedlings were transplanted in 2011. The rows have completely disappeared but no cultivation has been practiced for two years and yet it is a single species monoculture. On the other hand, the plants are nitrogen and moisture stressed and very few, tiny heads were produced.

Maintaining perennial forbs in rows and lightly cultivating between the rows early in the growing season may be one way that farmers can prevent overcrowding yet control invading perennials. Alternatively integrating animals which exhibit root scavenging behavior such as pigs in between rows may be one way of reducing unwanted spread while transporting nutrients across the landscape. If it is necessary to thin (and then weed) the stand within the row also, specialized cultivators or a companion cover crops may need to be developed.

CONCLUSION

Before investing heavily in new perennial crop domestication for a particular commodity and target environment, it would be worth thinking very strategically about what traits other than multi-year survival need to be captured. The breeders should begin discussions with agricultural engineers and agronomists to develop new kinds of equipment and procedures to enable farmers to fulfill the functions that the candidate species cannot do itself. The integration of companion species, equipment capable of root/rhizome "pruning", strip tillage and possibly mowing or cultivation within rows may prove to be necessary in many cases. At a more philosophical level, we wonder if it is possible to predict *a priori* what histories of natural selection are likely to have produced species with complex traits compatible with perennial agriculture.

Hypothesis 1: The life forms that dominate the native ecosystem in the regions and landscape positions targeted for grain production serve as useful models but may themselves not be useful for domestication as grains. Once a particular kind of life form (e.g. warm-season, sod-forming grasses in Kansas) outcompetes other life forms the major source of competition is intraspecific or at least intra-guild. This kind of competition leads to "arms races" which lead to the evolution of highly competitive morphologies such as trees with long trunks and prairie grasses with massive root systems (Craine, 2006). Undoing the effects of these arms races to produce a more efficient canopy or root community could be very difficult.

Non-dominant species have had to evolve strategies for escaping direct competition with the dominant life forms. Thus, in Kansas prairies, deep rooted forbs in the absence of disturbance may never be able to outcompete sod-forming grasses and become dominant, but they can survive as dormant tubers or seeds until a serious drought kills some grasses opening up niche spaces for new growth. The deep-root lifeform may be more useful in the context of agriculture in Kansas than the warm-season sod forming life form. On some landscape positions in this biome, *H. pauciflorus* and *H. maximiliani* can form large stands that are co-dominant with some grasses. *H. maximiliani* and *H. tuberosus* have been described as having allelopathic properties, this coupled with abundant adventitious growth in the presence of available resources tells us these species are highly competitive through a series of diverse mechanisms. However, in different environmental contexts, these species may never be able to form dense stands and thus may not be as competitive with other species…or itself.

Hypothesis 2: Climates or landscape positions where neither perennial nor annual agriculture is possible might seem to be sources of species well adapted to the kinds of stresses perennial crops in moderate-to-low input systems are likely to face. However, we suggest that species found mainly in these extreme locations may have experienced selection for stress tolerance rather than stress avoidance. Reduced growth rate is one way of surviving in a stressful environment, but this strategy is likely to be inimical to any form of agriculture. Evidence for this comes from switchgrass (*Panicum virgatum*) where cultivars derived from the wild upland ecotype showed less physiological stress under water and nitrogen limitations than lowland cultivars, but nevertheless were out-yielded by the lowland types in both stressed and unstressed treatments (Stroup *et al.* 2003).

Another strategy that approaches stress escape (fully realized in the annual habit) is highly plastic development. Such plants may be diminutive and asexual most years but capable of opportunistic growth and profuse flowering following disturbance or in favorable seasons when limitations have been temporarily relaxed (c.f. description above of *H. pauciflorus* in sod versus tilled ground). Stress tolerance/escape adaptations enable a species to survive but may not be useful in an artificial ecosystem designed to both take full advantage of available resources and to minimize seed yield instability from year to year and over the course of centuries.

The ideal perennial grain has adaptations for avoiding water and nutrient limitations, for example by exploiting deep soil moisture unavailable to other species, or by interacting with mutualists to obtain nitrogen or phosphorus in forms usable by plants. We suggest, therefore, that the best candidates for a region may be those capable of persisting and fruiting annually (though perhaps not as the dominant life form, according to Hypothesis 1) in the absence of agricultural inputs or nutrient-releasing disturbance but with the benefit of the soils and landscape positions where perennial agriculture is possible to be practiced.

These hypotheses are suggested as criteria for quickly identifying initial candidates and their adaptations to their environmental constraints; there is still no substitute for rigorous evaluation of all candidates for adaptation to the target range of stresses and input levels.

REFERENCES

Agarwal, A.K. 2007. Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines. *Progress in energy and combustion science.* 33(3): 233-271.

Atlagić, J., Dozet, B. & Škoric, D. 1995. Meiosis and pollen grain viability in *Helianthus mollis*, *Helianthus salicifolius*, *Helianthus maximiliani* and their F1 hybrids with cultivated sunflower. *Euphytica.* 81(3): 259-263.

Baxter, S.W., Davey, J.W., Johnston, J.S., Shelton, A.M., Heckel, D.G., Jiggins, C.D., & Blaxter, M.L. 2011. Linkage mapping and comparative genomics using next-generation RAD sequencing of a non-model organism. *PLoS ONE.* 6(4): e19315.

Blackman, B.K., Michaels, S.D. & Rieseberg, L.H. 2011. Connecting the sun to flowering in sunflower adaptation. *Molecular Ecology.* 20(17): 3503–3512.

Brooks, R.E. & Schofield, E.K. 1986. *Flora of the Great Plains*. Lawrence: University Press of Kansas.

Brown, M.J., Ferruzzi, M.G., Nguyen, M.L., Cooper, D.A., Eldridge, A.L., Schwartz, S.J. & White, W.S. 2004. Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection. *The American Journal of Clinical Nutrition*. 80(2): 396-403.

Burke, J.M., Tang, S., Knapp, S.J. & Rieseberg, L.H. 2002. Genetic analysis of sunflower domestication. *Genetics.* 161(3): 1257-1267.

Chapman, M.A. & Burke, J.M. 2012. Evidence of selection on fatty acid biosynthetic genes during the evolution of cultivated sunflower. *Theoretical and Applied Genetics.* 125(5): 897-907.

Chapman, M.A., Pashley, C.H., Wenzler, J., Hvala, J., Tang, S., Knapp, S.J. & Burke, J.M. 2008. A genomic scan for selection reveals candidates for genes involved in the evolution of cultivated sunflower (*Helianthus annuus*). *The Plant Cell Online.* 20(11): 2931-2945.

Clevenger, S. & Heiser, C.B. 1963. *Helianthus laetiflorus* and *Helianthus rigidus*—Hybrids or species? *Rhodora.* 65(762): 121-133.

Craine, J.M. 2006. Competition for nutrients and optimal root allocation. *Plant and Soil.* 285(1-2): 171-185.

Davey, J.W., Hohenlohe, P.A., Etter, P.D., Boone, J.Q., Catchen, J.M., Blaxter, M.L. 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics.* 12(7): 499–510.

DeHaan, L.R, Van Tassel, D.L. & Cox T.S. 2007. Perennial grain crops: a synthesis of ecology and plant breeding. *Renewable Agriculture and Food Systems*. 20(1): 5–14.

Edwards, S.V. 2013. Next-generation QTL mapping: crowdsourcing SNPs, without pedigrees. *Molecular Ecology.* 22(15): 3885-3887.

Ewers, R.M., Didham, R.K., Pearse, W.D., Lefebvre, V., Rosa, I.M.D., Carreiras, J.M.B., Lucas, R.M., & Reuman, D.C. 2013. Using landscape history to predict biodiversity patterns in fragmented landscapes. *Ecology Letters.* (in press).

Falconer, D.S. & Mackay, T.F.C. 1996. *Introduction to quantitative genetics* (4th edition). Longman Inc., Harlow UK.

Feng, J. & Jan, C.C. 2008. Introgression and molecular tagging of Rf 4, a new male fertility restoration gene from wild sunflower *Helianthus maximiliani* L.*Theoretical and Applied Genetics.* 117(2): 241-249.

Fu, Y.B., & Peterson, G.W. 2012. Developing genomic resources in two Linum species via 454 pyrosequencing and genomic reduction. *Molecular Ecology Resources*. *12*(3): 492-500.

Georgieva-Todorova, J. 1964. Interspecific hybridization in the genus Helianthus. In: *Interspecific hybridization of plants*, Bulgarian Academy of Science, Sofia, Georgieva-Todorova J. (ed), pp 135-82.

Glover, J.D., Reganold, J.P., Bell, L.W., Borevitz, J., Brummer, E.C., Buckler, E.S.,Cox, C.M., Cox, T.S., Crews, T.E., Culman, S.W., DeHaan, L.R., Eriksson, D., Gill, B.S., Holland, K., Hu, F., Hulke, B.S., Ibrahim, A.M.H., Jackson, W., Jones, S.S., Murray, S.C., Paterson, A.H., Ploschuk, E., Sacks, E.J., Snapp, S., Tao, D., Van Tassel, D.L., Wade, L.J., Wyse, D.L. & Xu, Y. 2010. Agriculture. Increased food and ecosystem security via perennial grains. *Science*. 328(5986): 1638–1639.

Göksoy, A.T., Demir, A.O., Turan, Z.M. & Dağüstü, N. 2004. Responses of sunflower (*Helianthus annuus* L.) to full and limited irrigation at different growth stages. *Field Crops Research*. 87(2-3): 167–178.

Harlan, J.R. & De Wet, J.M.J. 1977. Pathways of genetic transfer from *Tripsacum* to *Zea mays*. *Proceedings of the National Academy of Sciences*. 74(8): 3494-3497.

Heiser, C.B. 1965. Species crosses in *Helianthus* III. Delimitation of “section”. *Annals of the Missouri Botanical Garden*. 52(3): 364-370.

Hulke, B.S. & Wyse, D.L. 2008. Using interspecific hybrids with *H. annuus* L. In: *Proceedings of the 17th International Sunflower Conference*. Cordoba, Spain, pp 729–734.

Jan, C.C. & Ruso, J.A. 2000. Vigor reducing cytoplasms of perennial *Helianthus* species and their nuclear vigor restoration genes in cultivated lines. In: *Proceedings of the 22nd Sunflower Research Workshop*. January 18-19, 2000, Fargo, ND, pp 18-19.

Jan, C.C., Zhang, T.X., Miller, J.F. & Fick, G.N. 2002. Inheritance of rertility restoration for two cytoplasmic male sterility sources of *Helianthus pauciflorus* (rigidus) Nutt. *Crop science*. 42(6): 1873-1875.

Kantar, M., Betts, K., Stupar, B., Hulke, B. & Wyse, D. 2010. The development of perennial sunflower for wildlife and food uses. Poster presented at the 8th Annual Sclerotinia Initiative Meeting, January 20-22, 2010, Bloomington, MN. (Available at www.sunflowernsa.com/research/research-workshop/documents/Kantar_Perennial_WildlifeFood_10.pdf)

Kantar, M.B., Betts, K., Michno, J.M., Luby, J.J., Morrell, P.L., Hulke, B.S., Stupar, R.M. & Wyse, D.L. 2014. Evaluating an interspecific *Helianthus annuus* x *Helianthus tuberosus* population for use in a perennial sunflower breeding program. *Field Crops Research*. 155:254–264.

Kawakami, T., Morgan, T.J., Nippert, J.B., Ocheltree, T.W., Keith, R., Dhakal, P. & Ungerer, M.C. 2011. Natural selection drives clinal life history patterns in the perennial sunflower species, *Helianthus maximiliani*. *Molecular Ecology*. 20(11): 318–2328.

Kays, S.J. & Nottingham, S.F. 2007. Biology and chemistry of Jerusalem artichoke*: Helianthus tuberosus L*. CRC press, Baton Rouge, Louisiana.

Kell, D.B. 2011. Breeding crop plants with deep roots: their role in sustainable carbon, nutrient and water sequestration. *Annals of Botany*. *108*(3): 407-418.

Kowalski, R. 2004. Evaluation of chemical composition of some *Silphium* L. species seeds as alternative foodstuff raw materials. *Polish Journal of Food and Nutrition Sciences*. 13(4): 349-354.

Lesica, P. & Allendorf, F.W. 1999. Ecological genetics and the restoration of plant communities: Mix or match? *Restoration Ecology*. 7(1): 42–50

Manel, S., Joost, S., Epperson, B.K., Holderegger, R., Storfer, A., Rosenberg, M.S., Scribner, K.T., Bonin, A., Fortin, M.J. 2010. Perspectives on the use of landscape genetics to detect genetic adaptive variation in the field. *Molecular Ecology*. 19(17): 3760-3772.

Marcel-les-Valence, F. 2005. Pruning intensity and fruit load influence vegetative and fruit growth in an early-maturing peach tree (cv. Alexandra). *Fruits*. 60(2): 133-142.

Muellenborn, C., Krause, J.H. & Cerboncini, C. 2011. Analysis of differential transcript expression reveals time-dependent leaf responses to *Sclerotinia sclerotiorum* in wild and cultivated sunflower. *Plant Molecular Biology Reporter*. 29(3): 597-608.

Narum, S.R., Buerkle, C.A., Davey, J.W., Miller, M.R. & Hohenlohe, P.A. 2013. Genotyping-by-sequencing in ecological and conservation genomics. *Molecular Ecology*. 22(11): 2841–2847.

Otto, S.P. & Lenormand, T. 2002. Resolving the paradox of sex and recombination. *Nature Reviews Genetics. 3*(4): 252-261.

Poland, J., Endelman, J., Dawson, J., Rutkoski, J., Wu, S., Manes, Y., Dreisigacker, S., Crossa, J., Sanchez-Villeda, H., Sorrells, M. & Jannik, J.L. 2012. Genomic selection in wheat breeding using genotyping-by-sequencing. *The Plant Genome Journal.* 5(3): 103-113.

Rashid, K.Y. & Seiler, G.J. 2004. Epidemiology and resistance to *Sclerotinia* head rot in wild sunflower species. In *Proceedings of the 16th International Sunflower Conference*. Fargo, ND, August 29-September 4, 2004. pp. 751-754.

Rashid, K.Y. & Seiler, G. 2005, February. Epidemiology and resistance to Sclerotinia head rot in wild sunflower species. In: *Proceedings of the13th International Sclerotinia Workshop*. Monterey, California, USA, 12-16 June, 2005

Ratcliff, W.C., Denison, R.F., Borrello, M. & Travisano, M. 2012. Experimental evolution of multicellularity. *Proceedings of the National Academy of Sciences.* 109(5): 1595-1600.

Reagon, M. & Snow, A.A. 2006. Cultivated *Helianthus annuus* (Asteraceae) volunteers as a genetic "bridge" to weedy sunflower populations in North America. *American Journal of Botany.* 93(1): 127-133.

Rogers, C.E., Thompson, T.E. & Seiler, G.J. 1982. *Sunflower species of the United States*. National Sunflower Association, Bismarck, North Dakota, USA.

Sadras, V.O. & Egli, D.B. 2008. Seed size variation in grain crops: Allometric relationships between rate and duration of seed growth. *Crop Science.* 48(2): 408.

Seiler, G.J. 1992. Utilization of wild sunflower species for the improvement of cultivated sunflower. *Field Crops Research.* 30(3): 195-230.

Seiler, G.J. & Brothers, M.E. 1999. Oil concentration and fatty acid composition of achenes of *Helianthus* species (Asteraceae) from Canada. *Economic Botany.* 53(3): 273-280.

Sideli, G.M., Schierenbeck, K.A. &Van Tassel, D.L. 2013. Evaluation of interspecific hybrids between wild perennial sunflower and domesticated sunflower for crop use with limited irrigation. Abstract presented at the 24th Graduate Student Meeting of the California Botanical Society, April 14 2013, Berkely California. (Available at www.calbotsoc.org/Symposium_Schedule_files/CentennialProgram_04092013web.pdf).

Siikamäki, P. 1999. Developmental instability in hybrids between *Lychnis viscaria* and *Lychnis alpina* (Caryophyllaceae). *American Journal of Botany.* 86:1683-1686

Sillanpää, M.J. 2011. On statistical methods for estimating heritability in wild populations. *Molecular Ecology.* 20(7): 1324–1332.

Stroup, J.A. Sanderson, M.A., Muir, J.P., McFarland, M.J., & Reed, R.L. 2003. Comparison of growth and performance in upland and lowland switchgrass types to water and nitrogen stress. *Bioresource Technology.* 86(1): 65-72.

Timme, R.E., Simpson, B.B. & Linder, C.R. 2007. High-resolution phylogeny for *Helianthus* (Asteraceae) using the 18S-26S ribosomal DNA external transcribed spacer. *American Journal of Botany.* 94(11): 1837-1852.

Travisano, M. & Shaw, R.G. 2013. Lost in the map. *Evolution.* 67(2): 305-314.

van het Hof, K.H., West, C.E., Weststrate, J.A. & Hautvast, J.G. 2000. Dietary factors that affect the bioavailability of carotenoids. *The Journal of Nutrition.* 130(3): 503-506.

Van Tassel, D.L., DeHaan, L.R. & Cox, T.S. 2010. Missing domesticated plant forms: can artificial selection fill the gap? *Evolutionary Applications. 3*(5-6): 434-452.

Vear, F. 2011 Helianthus. In: *Wild Crop Relatives: Genomic and Breeding Resources*, Chittaranjan K. (Ed.) Springer, Berlin, pp 161-170.

Velasco, L., Pérez-Vich, B. & Fernández-Martínez, J.M. 2004. Evaluation of wild sunflower species for tocopherol content and composition. *Helia.* 27(40): 107-112.

Vollmann, J. & Rajcan, I. 2010. Oil crop breeding and genetics. In: *Handbook of Plant Breeding* Volume 4, Oil Crops. Johann Vollmann and Istvan Rajcan (Eds). Springer New York, 2010. 1-30.

Weaver, J.E. & Stoddart, L.A. 1935. Response of the prairie to the great drought of 1934. *Ecology*. 16(4): 612-629.

Xia, Y. & Larock, R.C. 2010. Vegetable oil-based polymeric materials: synthesis, properties, and applications. *Green Chemistry*. *12*(11): 1893-1909.

Zimmer, D.E. & Rehder, D. 1976. Rust resistance of wild *Helianthus* species of the North Central United States. *Phytopathology*. 66(2): 208.

10

DOMESTICATION OF *LEPIDIUM CAMPESTRE* AS PART OF MISTRA BIOTECH, A RESEARCH PROGRAMME FOCUSED ON AGRO-BIOTECHNOLOGY FOR SUSTAINABLE FOOD

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ABSTRACT

Several important challenges are facing agriculture. In the stride towards lowering the negative environmental impact of food production while maintaining and increasing the production, both basic farming practices and novel technologies are important tools. The use of biotechnology in breeding, however, is not uncontroversial. The criticism has many angles and often relates to the applications brought forward by large, multinational companies, and farmers growing dependence on these companies when it comes to seeds. Questions are being raised about ethical acceptability,

and about the health and environmental impacts. A general aversion to what is often referred to as the “industrialization” of agriculture, and to “unnaturalness”, also emerges in the debate.

In the research programme referred to as *Mistra Biotech*, we include both philosophy and natural and social sciences. The overall goal is to facilitate production systems that are sustainable from ecological, social and economic perspectives.

The objectives of the programme include developing:

- new elite plant lines that have benefits for consumers, farmers, the food industry and the environment;
- agribiotechnology tools that are important for achieving new product qualities, healthier crops and livestock, and for solving environmental problems in agriculture;
- basis for sustainable production systems that contribute to increased competitiveness in Swedish agriculture and food production;
- tools for ethical scrutiny of agricultural biotechnology that combine high demands on safety with encouragement of innovations;
- basis for improved regulatory approaches and private-public relationships.

The programme includes six component projects, in which domestication of a new biennial oilseed crop *Lepidium campestre* (field cress) is a major research focus. Questions we will try to answer within this programme include: Can biotechnology be used to improve crops which mitigate climate change or benefit the environment? What potential is there to commercialize such a crop? How would the consumers react to products made from it? Can breeding technology be improved further? Why does the market for genetically improved plant and animal materials look the way it does? What ethical concerns does the use of biotechnology raise? And how do all these issues feed into future agricultural systems? The results are integrated in the synthesis project called the Centre for Agriculture and Food Systems Analysis and Synthesis (AgriSA).

Here we emphasise the research within Component Project1, with a focus on the domestication of field cress.

PLANT BIOTECHNOLOGY FOR INNOVATIVE PRODUCTS (Component Project 1)

Domestication of *Lepidium campestre* through GM and non-GM approach

The demand of plant oils for food and biodiesel is expected to increase steadily in the coming 20 years. However, the potential of increasing production from the existing oilseed crops is limited. For example, in Sweden, the only economically viable oilseed crop is winter rapeseed, but it can only be grown in the southern part of the country.

We are pursuing a fast-track domestication of *Lepidium campestre* (field cress), a biennial *Brassicaceae* species, into a combined novel oil- and catch crop. Late professor Arnulf Merker at the Swedish University of Agricultural Sciences (SLU) identified *L. campestre* as a promising species for domestication - a high seed yielding plant (5-6 tonnes/ha, which is higher than the average yield of winter oilseed rape) with an upright stature and synchronous flowering. Moreover, it is biennial with a potential to be a perennial crop. As a cover crop, it is sown under cereal crops during spring, and seeds are harvested the following year; thus reducing nutrient leaching and tillage - a major factor that causes groundwater contamination, especially under intensive use of nitrogen based fertilizers and tillage. Planting biennial or perennial crops and use of cover crops could alleviate this problem. Field cress could be a promising crop species that can fit into such cropping system: a system that is very limited in Sweden. Field cress is also cold-hardy and can be successfully grown in the northern parts of Sweden. However, some of its properties must be altered in order for the plant to be an economically viable oil crop.

In the new cereal-oilseed cropping system being developed, we are using field cress and barley (*Hordeum vulgare L.*) as a model. For genetic improvement of field cress we are using both conventional methods as well as genetic engineering for speeding up the breeding process and for comparing the breeding efficiency of the two methods. The main targeted traits are oil content, oil quality, seed yield and pod shatter. Pod shatter, which is the dropping of seeds before harvest, causes huge losses in seed yield. A number of transgenic lines with genes for improving the aforementioned traits have been recovered and are currently under screening to identify homozygous lines. The preliminary results have shown increased oil content in some transgenic lines, and an increased level of the healthy oleic acid (omega-9), a monosaturated fatty acid in others. The transgenic lines with improved shatter-resistance genes are under evaluation.

To provide more populations for conventional domestication and breeding, we collected a large number of field cress populations from several locations in Sweden in addition to a large number of accessions obtained from various gene banks and botanical gardens around the world. The screening in the greenhouse and outdoors has been focused on the identification of genotypes with one or more of the following desirable traits: low pod-shattering, resistant to major diseases and pests, high seed yield, high oil content, large seed size, synchronised maturity, well-developed root systems and perenniality. Seed oil content and composition analyses have shown a wide variation in oil content, but small variations in fatty acid composition among different accessions with linoleic acid (polyunsaturated) as the highest of all fatty acids. Among the mono-unsaturated fatty acids, erucic acid was dominant, followed by oleic acid. The seed also contains tocopherols and cholesterol. Promising genotypes with various combinations of these traits have now been selected for further evaluation. Intra-and inter-specific hybridizations have also been made. In the case of intra-specific hybridization, crosses have been made between genotypes with elevated levels of oil content, between high seed yielding genotypes, as well as between relatively low pod-shattering genotypes in an attempt to create superior genotypes. Hybrids that are superior to their parents are under further evaluation. Inter-specific hybridizations were made between field cress and other species in the genus *Lepidium* to transfer desirable traits from the latter to the former. The most successful inter-specific hybridization was between field cress and *Lepidium heterophyllum*, a perennial close relative of field cress. The F_1 hybrids produced from these species were perennial and showed very strong hybrid vigour with a significantly higher seed yield and a root system superior to those of both parents. Interesting lines have been selected from the F_2 populations for further breeding. Interspecific hybridizations were also made between field cress and *Lepidium draba* to transfer shatter-proof genes from *L. draba* to field cress; and between field cress and *Lepidium graminifolium* to increase the oil content in field cress. Overall, a significant increase in oil content and seed yield, a significant decrease in pod shattering, and improvement in root systems are all highly promising developments, as is the progress in developing perennial field cress.

At Component Project 1 we are also investigating and improving other crops and traits:

Improve barley and potato: Here we focus on improving nitrogen uptake and pathogen resistance using various genetic technologies. One of the technologies used is site directed mutagenesis, which consists of a number of techniques including TALEN (transcription activator-like effector nuclease) to improve disease resistance in barley and potato. So far, we have synthesised four nucleases for potato and transfection of potato with these nucleases is under evaluation. For improving nitrogen uptake in barley, the transgenic barley lines with introduced genes responsible for root uptake of amino acids were field tested, mainly for producing seeds for further evaluation. The seeds from the field trial are currently under evaluation in controlled growing conditions in a greenhouse for their nitrogen uptake ability.

Starch quality analysis: We have investigated the fine molecular structure of the amylopectin isolated from various genotypes of barley and have found that a mutation linked to starch biosynthesis results in a modified amylopectin structure. We are now investigating how different molecular units in amylopectin are interconnected in these barley samples. A certain category of glucose chains, building up the amylopectin molecule, is thought to play a role in cluster interconnection, and thereby in the compiling of domains. Other categories of shorter chain-lengths are believed to interconnect smaller amylopectin building blocks. More information on the fine structure of amylopectin will improve our understanding of the relationship between starch structure and several functional properties. These relationships will be studied during the coming year.

High amylose potato: Potato starch is usually made up of 25 percent amylose and 75 percent amylopectin. By turning off two genes controlling amylopectin biosynthesis, we could increase the amylose level. The field trial on the transgenic lines shows that high amylose potato had reduced starch content and an increased tuber yield. The preliminary analysis shows that the starch content is about 35–70 percent of the non-transgenic control. Further studies on circumventing the starch yield drag through crossing are underway. Transcriptome analyses will be carried out to elucidate molecular mechanisms underlying the starch yield drag.

OTHER PROJECTS WITHIN MISTRA BIOTECH

Novel molecular breeding tools (Component Project 2)

The majority of economically important traits in crops and livestock, such as product yield, product quality and disease resistance are complex traits governed by many genes and environmental factors. Traditional breeding approaches have used pedigree information and statistical tools to estimate the proportion of variation that is due to heritable factors, but treated the genome as a “black box”. Today new technologies facilitate the sequencing at a fraction of the original costs. We will be providing methods and tools for the use of whole genome sequence data in breeding – that is, selecting plants and animals using information about their entire DNA sequence instead of looking at specific genes. Additionally, we will be investigating the potential to use information about proteins, the genetic product, in breeding. The gain here is the ability to screen for and select suitable plants and animals at an early stage in the breeding process.

Ethics (Component Project 3)

The debate about ethical issues in biotechnology and its applications is very polarized. Some people are against, some in favour, and these views are often firmly held. Despite the large literature on ethics of technology in general, there is a shortage of studies carried out in close collaboration with the scientists developing actual technologies. Therefore, much of the debate is insufficiently informed by recent developments and rather sweeping in character. Also, few applications of ethical technology assessment involve new biotechnologies, and even fewer take into account the potentially positive environmental and health impacts of agricultural applications of biotechnology in a systematic way. We hope to provide a structured method of making this debate less polarized, allowing everyone to better understand each other’s arguments. We will also investigate what a “precautionary” approach might involve in the context of agricultural biotechnology And we will study some concepts that are common in the public debate, but which are sometimes cursorily treated in the scholarly discourse, such as naturalness and sustainability.

Consumer attitudes and behaviour (Component Project 4)

What are the driving forces behind attitudes and behaviours when it comes to food produced using agricultural biotechnology? What is our perception of risks and trust? We hope to reach a better understanding of the underlying consumer-related issues that will play an essential role in the uptake and use of any application of agricultural biotechnology in Sweden. Our first results come from a meta-study combining the results from over 1 600 questions in 241 different studies in 58 regions. The study shows that previous conclusions on the Europeans' negative attitude towards GM food might be the result of slightly different questions having been asked in Europe compared with other countries.

Driving forces behind applications of biotechnology (Component Project 5)

The economic and regulatory environment in which firms operate has a direct effect on their ability to produce and to adopt new technologies. Firms will produce innovations when they have the ability to commercialize, to sell a product or service at a profit. The profitability of an innovation depends on the degree to which they are able to capture the economic rents generated by their innovations. Farmers will adopt innovations for similar reasons. However, the ability of agrifood value chain to distribute the benefits from consumers, to farmers, from retailers to processors and to biotech firms is the challenge. The competition along the agrifood value chain, the governance of transactions and the regulatory framework are the determining factors and the subject of research in this team.

We analyse the structure and governance of the Swedish agrifood system and the national and international regulatory environments. We also explore Sweden's capacity to produce and distribute innovative products and processes, constraints on this capacity, and the impact of all this on the Swedish economy. We plan to provide a synthesis which will be part of the basis for discussion of policy recommendations. The results will be relevant to actors in the primary agriculture sector, the biotech industry, and other stakeholders in the processing and distribution agrifood industry.

Centre for agriculture and food systems analysis and synthesis (Component Project 6, AgriSA)

The work in AgriSA focuses on whole production systems and stretches across disciplines within the human, agricultural, natural, and social sciences. The aim of this work is to understand and facilitate the implementation of sustainable food production using biotechnology as a tool. AgriSA is the hub where the information and results from all Mistra Biotech projects are processed and where overall syntheses are made and communicated to stakeholder groups. The work will include, among other things, scenario development, lifecycle assessment and cost-benefit analysis. Scientists from all the Mistra Biotech projects are working together in AgriSA. The work will also involve stakeholders and experts on food production systems and methods of system analysis.

Issues currently in focus in AgriSA:

1. What is included in the concept of sustainability in relation to green biotechnology?
2. Ecological consequences of biotechnology in plant and animal breeding.
3. Field trials, communication and relations with producers and consumers.
4. Sustainability assessments of different production systems.
5. Goal conflicts – can biotechnology help to solve conflicts between different environmental goals and between environmental goals and other goals of the society?
6. Ability to improve the nutritional value of food with plant and animal breeding

Mistra Biotech

Mistra Biotech involves over 50 researchers. Most of them work at SLU, while some work at the Royal Institute of Technology, Lund University, and other academic institutions. Mistra Biotech is funded by the Swedish Foundation for Strategic Environmental Research (Mistra) and SLU. Many companies, agencies and organizations also support the programme with their knowledge, experience and valuable feedback.

11

AGRICULTURE REDESIGN THROUGH PERENNIAL GRAINS: CASE STUDIES

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ABSTRACT

Multiple purpose perennial crops are potentially the most adoptable by farmers and of most value to society, as means to provide grain, forage for livestock systems, recycling of nutrients to protect water quality, and build soils. This paper examines the services provided by two perennial grain crops as case studies: perennial wheat and semi-perennial pigeon pea. In Michigan, intermediate wheatgrass provided significant benefit to environmental services when investigated in field experimentation, especially through reducing leaching of nitrogen to almost nil. The results were consistent with perennial wheat as providing valuable environmental services, particularly in places with steep, marginal lands, such as Nepal. In Africa, diversification with legumes has a long history, and is the basis of the natural regeneration of fertility in extended bush fallows which dominated agricultural production until recent decades. A new type of perenniation is urgently needed, one where semi-perennial legume food crops are integrated with cereals and tuber crops. Improved varieties of legumes have tended to emphasize short-duration growth types with large grain to shoot ratios (high harvest index); this has come at the cost of extended vegetative cover which can double or triple photosynthesis and nitrogen fixation potential. Judicious combinations of short-duration food legumes (soybean, cowpea, groundnut and bean) and shrubby or viney grain legumes (pigeon pea, mucuna and climbing bean) with cereal crops is a promising way forward, to sustainably intensify production while gaining desirable perennial traits. A 'doubled up legume' system of pigeon pea grown in mixtures with short-statured food legumes, where pigeon pea is ratooned to grow a second year as an intercrop with maize has doubled fertilizer efficiency compared to sole maize, based on hundreds of participatory research actions on-farm in Malawi. Over 10 000 farmers are now pursuing innovations with various combinations of improved pigeon pea germplasm and integrated crop, soil and residue management. Participating villages have demonstrated, measurable improvements in yield stability and child nutrition. This is some of the emerging evidence that perennial grains can help communities overcome degraded soils and build family health for a resilient future.

Keywords: participatory research, agricultural systems, pigeon pea, perennial wheat, doubled up legumes

INTRODUCTION

The purpose of this paper is to illustrate the potential of two perennial grain crops — (i) perennial wheat (*Triticum aestivum x Thinopyrum elongatum*) and (ii) a semi-perennial legume crop, pigeon pea (*Cajanus cajan*) — to address critical production issues. The contribution of perennial wheat is explored using intermediate wheatgrass in Michigan. A doubled up legume system with pigeon pea is explored in Malawi. The results are used to illustrate how perennial grains can improve ecosystem sustainability and family health.

PERENNIAL WHEAT

A comprehensive, 4-year field study conducted in southwest Michigan is quantifying environmental services associated with perennial cereals, including high nitrogen retention, pure water quality, and soil building (Culman *et al.* 2013). Perennial wheat is a novel cereal crop under development through two different pathways. One pathway is the domestication of a perennial relative of annual wheat (*Triticum aestivum*), a forage grass called intermediate wheatgrass (*Thinopyrum intermedium)*. Another pathway that plant breeders are pursuing is to cross perennial grasses with annual wheat to develop a perennial form of wheat. Both pathways have the potential to produce multi-purpose crops; that is, crops that can be grown for grain or fodder and provide environmental benefits (Glover *et al.* 2010).

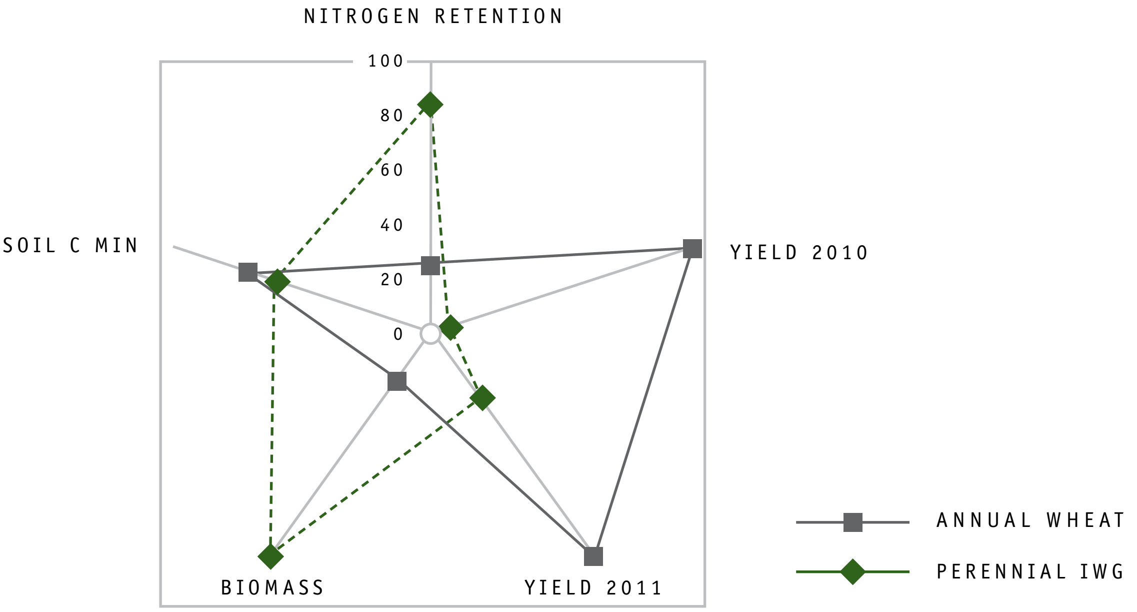
Dozens of lines of perennial wheat have been developed by plant breeders through repeated crossing of annual wheat with perennial grasses including intermediate wheatgrass and *Thinopyrum elongatum*. The new crop is under selection, as breeders choose lines that have a perennial growth habit (with post-sexual reproduction regrowth), while exhibiting grain characteristics similar to that of the annual wheat parent. The majority of the genetic makeup of this new crop is that of annual wheat, at least among the lines being developed at Washington State University by Drs. Stephen Jones and Kevin Murphy (Murphy *et al.* 2009).

Since 2007, field studies have been underway in Michigan at the W.K. Kellogg Biological Station, evaluating intermediate wheat grass (IWG) and perennial wheat lines for production potential and other ecosystem services such as soil building properties. Grain yields remain low to moderate, from about 0.3 to 1.8 mg per ha grain yield of perennial cereals relative to 2 to 4 mg per ha from annual wheat (Jaikumar *et al.* 2012). From an initial, 2 year assessment, IWG produces modest to almost zero grain yield and is a vigorous, true perennial grass that can be grown as a forage crop as it is highly productive and of good quality fodder (Figure 1), and it has substantial environmental benefits (such as reducing nitrogen leaching to almost undetectable amounts). (See Culman *et al.* 2013 for the first report on this novel cropping system). The root growth and biomass belowground of IWG is consistently five-fold higher, relative to annual wheat (C. Sprunger, unpublished data).

Perennial wheat has the advantage that it produces a crop that is recognizable as wheat, so the grain can be sold in an already-existing market, and there is the potential for growers to use it as a dual purpose crop whereby farmers who have livestock graze it during the spring, then plants are allowed to grow back and grain harvest occurs later that same year. Farmers reap an immediate return and have the added benefit of gaining from its long-term impact: large root systems that we predict will improve soil-organic matter, carbon sequestration, and water quality. There is considerable evidence that water quality is improved markedly by growing a crop of IWG, with 80 percent or higher retention of nitrogen in the soil-crop system, reducing nitrate leaching to almost zero through water loss pathways, as indicated by field experiments in the Snapp lab at Michigan State University (Figure 1) (Culman *et al.* 2013).

Somewhat surprisingly, there is limited evidence from these field experiments that IWG or perennial wheat lines have been effective at supporting rapid gains in soil carbon. The active soil carbon pool and nitrogen recycling is indeed markedly improved by IWG, but total soil carbon has been slow to respond in the initial years of these long-term perennial grain experiments.

FIGURE 1. BASED ON DATA PRESENTED IN CULMAN *ET AL.* (2013) COMPARING THE ECOSYSTEM SERVICES SUPPORTED BY PERENNIAL GRAIN IWG WITH THOSE PRODUCED BY ANNUAL WHEAT IN A FIELD TRIAL CONDUCTED AT KELLOGG BIOLOGICAL STATION, MSU, IN SOUTHWEST MICHIGAN



Soil C respiration measurements are consistent with active soil C pools being modestly enhanced in the presence of IWG, by about 15 percent relative to annual wheat (Figure 1). But no effect of a perennial grain (relative to annual wheat) has been observed for soil carbon sequestration pathways, as indicated by soil permaginate oxidizable carbon measurements (Culman *et al.* 2013). The almost complete lack of overlap between the prototype perennial grain IWG and annual wheat shows that the benefits of annual wheat are primarily grain yield as yet, whereas IWG has considerable fodder and environmental services and is not yet a significant producer of grain.

Taken together, the results are consistent with IWG as fitting well into a dairy or cattle farm. In contrast, perennial wheat shows potential as a cover crop to grow in marginal areas of the farm to protect fragile areas such as along riverbeds, but the unique property of perennial wheat is that, in addition to soil conservation, these new genotypes can also produce yields of grain and fodder. These results have implications for other regions of the world, such as Western Nepal, where wheat is the dominant crop and where its production as an annual crop requires excessive amounts of labour, provides too few additional benefits (e.g. to livestock), and is highly variable due to variable weather. Perennial wheat offers a range of opportunities for overcoming the challenges of annual wheat production (Figure 2).

FIGURE 2. DR. DHRUBA THAPA, A WHEAT BREEDER WITH THE NEPAL AGRICULTURAL RESEARCH COUNCIL, HIGHLIGHTS THE STRONG REGROWTH OF SOME OF HIS PERENNIAL WHEAT HYBRIDS

He believes perennial wheat will improve the lives of women farmers, increase wheat yields and quality, and meet multiple farming system needs.

FIGURE 3. TALLER, SLOWER GROWING PIGEON PEAS COMPLEMENT LOWER- AND FASTER-GROWING GROUNDNUTS, WHICH ARE READY FOR HARVEST SEVERAL WEEKS BEFORE PIGEON PEAS MATURE

SEMI-PERENNIAL PIGEON PEA

Another case study, highly relevant to tropical farming systems, is that of the perennial legume species, pigeon pea (*Cajanus Cajan*) (Snapp *et al.* 2003). It is often grown as an annual but traditional cropping systems still involve production of pigeon pea as a short-lived perennial (two to three years). It is a tropical legume that grows as a shrubby semi-perennial crop, and demonstrates a unique set of multi-functional properties, which is being documented in Malawi, southern Africa (Glover *et al.* 2012; Snapp *et al.* 2010). Farmers have long relied on pigeon pea to provide flexible options for livestock feeding and crop production, enhancing flexibility and productivity of the entire farm system. Recent genetic improvements in pigeon pea include new varieties that are broadly adapted to diverse environments. Farmer research groups are testing agronomic innovations through participatory research approaches that promote farmer innovation (Bezner-Kerr *et al.* 2007).

Over 9 000 farm families have newly adopted pigeon pea in the northern Ekwendeni region of Malawi (Snapp *et al.* 2010). Farmer education through participatory action research has promoted experimentation, which includes planting arrangements, crop residue management, and improved fodder for dairy cattle. Because parts of Africa's climate are tropical, farmers can incorporate pigeon pea into cash crops as they can grow for one to three years. After it is harvested, growers can cut it back and use the vegetative material as fodder for livestock or to improve soil fertility, and can sell the peas for profit or use them as a protein-rich food.

Agro-ecology training of farmers has supported local testing of a range of perennial-diversified options, and older, drought tolerant crops such as land races of sorghum are being grown as intercrops with pigeon pea and with other grain legumes (e.g. doubled up legume mixtures of pigeon pea and groundnut or pigeon pea and soybean), and in maize mixed systems (Figures 3 and 4). Growing pigeon pea and doubled up legume systems (with pigeon pea intercropped with soybean or groundnut) has been tested in Kenya and Malawi, with growing evidence that they can substantially enhance production of nutrient-enriched grain compared to sole cropped maize (Snapp and Silim, 2002; Snapp *et al.* 2010; Figure 5). Pigeon pea has a deep root system and leafy residues that enhances soil fertility for consistent yield gains in rotated crops such as maize, as well as supporting production of grain directly.

FIGURE 4. PIGEON PEAS PROVIDE INTERCROPPING OPPORTUNITIES FOR FARMERS

Because of their slow growth rates in the first year, they do not compete aggressively with faster growing legumes such as groundnuts. As they regrow in the second season, they can compete with more aggressive crops such as maize.

DOUBLED-UP LEGUME SYSTEM

- increases plants' efficiency of fertilizer use
- improves yield of protein-rich grains
- decreseas labour requirements
- improves families' diets

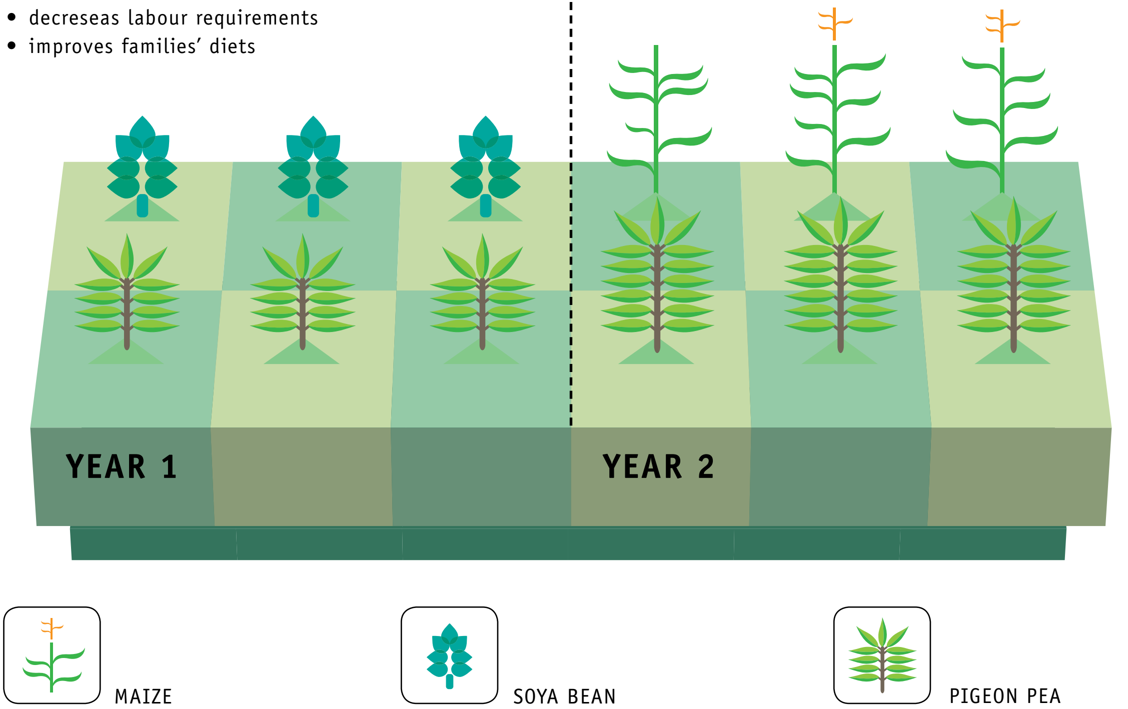
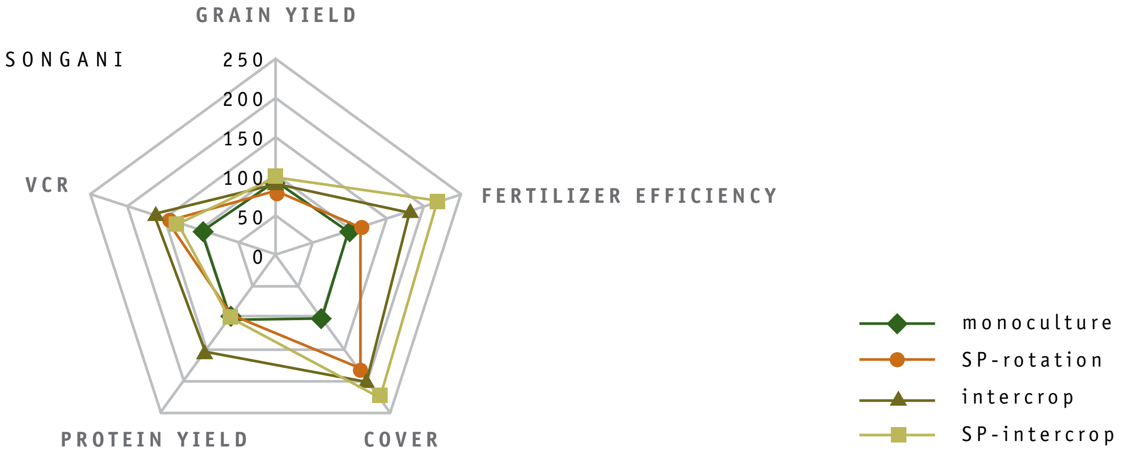


FIGURE 5. SHRUBBY PIGEON PEA INTERCROPS (SP-INTERCROP) AND SHRUBBY PIGEON PEA ROTATIONS (SP-ROTATIONS) DECREASE FERTILIZER REQUIREMENTS; IMPROVE THE VALUE COST RATIO (VCR), FERTILIZER USE EFFICIENCY, AND PROTEIN YIELDS; INCREASE CARBON AND NITROGEN ASSIMILATION AND PHOSPHORUS AVAILABILITY; AND PROVIDE GREATER COVER THAN MONOCULTURE MAIZE



Source: Snapp *et al.* 2010

Long-term field experiments and on-farm monitoring in Malawi have documented the unique ability of these polyculture perennial systems to support gains in soil organic carbon, nitrogen, and water use efficiency and adaptation of crops to climate variability. These are some of the lines of evidence suggesting that perennial crops provide farmers with important new options as a foundation for sustainable intensification of smallholder production in Africa. Further, we predict that the environmental benefits that are associated with perennial grains will prove vital to smallholder farmers' ability to buffer crop production and cope with the on-going variability that is associated with weather patterns in sub-Saharan Africa. Global warming is predicted to induce enhanced variability in the near future, so coping with droughts, rising temperatures, and variable weather will be even more important in the coming decades. The examples presented here illustrate how perennial grains can be used in combination with judicious inputs to provide a sound foundation for wise resource use, to protect the environment, and to support sustainable production in a rapidly changing world.

REFERENCES

Bezner-Kerr, R., Snapp, S.S., Chirwa, M., Shumba, L. & Msachi, R. 2007. Participatory research on legume diversification with Malawian smallholder farmers for improved human nutrition and soil fertility. *Experimental Agriculture.* 43: 437-453.

Culman, S.W., Snapp, S.S., Ollenburger, M., Basso, B. & DeHaan, L.R. 2013. Soil and water quality rapidly responds to the perennial grain Kernza wheatgrass. *Agronomy Journal.* 105: 735-744.

Glover, J.D., Reganold, J.P., Bell, L.W., Borevitz, J., Brummer, E.C., Buckler, E.S., Cox, C.M., Cox, T.S., Crews, T.E., Culman, S.W., DeHaan, L.R., Eriksson, D., Gill, B.S., Holland, J., Hu, F., Hulke, B.S., Ibrahim, A.M.H., Jackson, W., Jones, S.S., Murray, S.C., Paterson, A.H., Ploschuk, E., Sacks, E.J., Snapp, S., Tao, D., Van Tassel, D.L., Wade, L.J., Wyse, D.L. & Xu, Y. 2010. Increased food and ecosystem security via perennial grains. *Science.* 328: 1638-1639.

Glover, J.D., Reganold, J.P & Cox, C.M. 2012. Plant perennials to save Africa's soils. *Nature.* 489: 360-361.

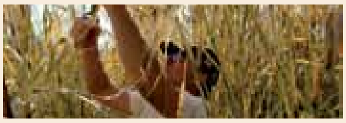
Jaikumar, N.S., Snapp, S.S., Murphy, K. & Jones, S.S. 2012. Agronomic assessment of perennial wheat and perennial rye as cereal crops. *Agronomy Journal.* 104: 1716-26.

Murphy, K. M., Hoagland, L.A., Reeves, P.G., Baik, B.-K. & Jones, S.S. 2009. Nutritional and quality characteristics expressed in 31 perennial wheat breeding lines. *Renewable Agriculture and Food Systems.* 24: 285-292.

Snapp, S.S., Blackie, M.J., Gilbert, R.A., Bezner-Kerr, R. & Kanyama-Phiri, G.Y. 2010. Biodiversity can support a greener revolution in Africa. *Proceedings of the National Academy of Sciences USA*. 107: 20840-20845.

Snapp, S.S., Jones, R.B., Minja, E.M., Rusike, J. & Silim, S.N. 2003. Pigeonpea for Africa: A versatile vegetable--and more. *HortScience.* 38: 1073-1078.

Snapp, S.S. & Silim, S.N. 2002. Farmer preferences and legume intensification for low nutrient environments. *Plant and Soil.* 245: 181-192.

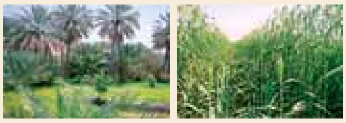


12 From genetics and breeding to agronomy to ecology

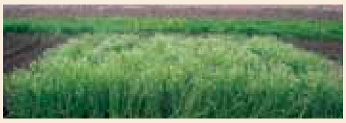
13 Economics and system applications for perennial grain crops in dryland farming systems in Australia

14 From field to table: perspectives and potential for fruit domestication

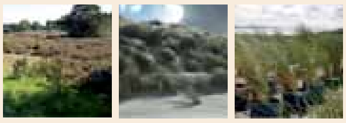
15 Development and marketing of perennial grains with benefits for human health and nutrition



16 Intercropping of legumes with cereal crops in particular with the perennials to enhance forage yields and quality



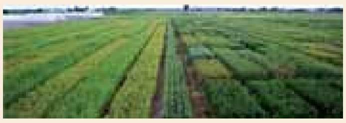
17 Development of continuous living cover breeding programs to enhance agriculture’s contribution to ecosystem services



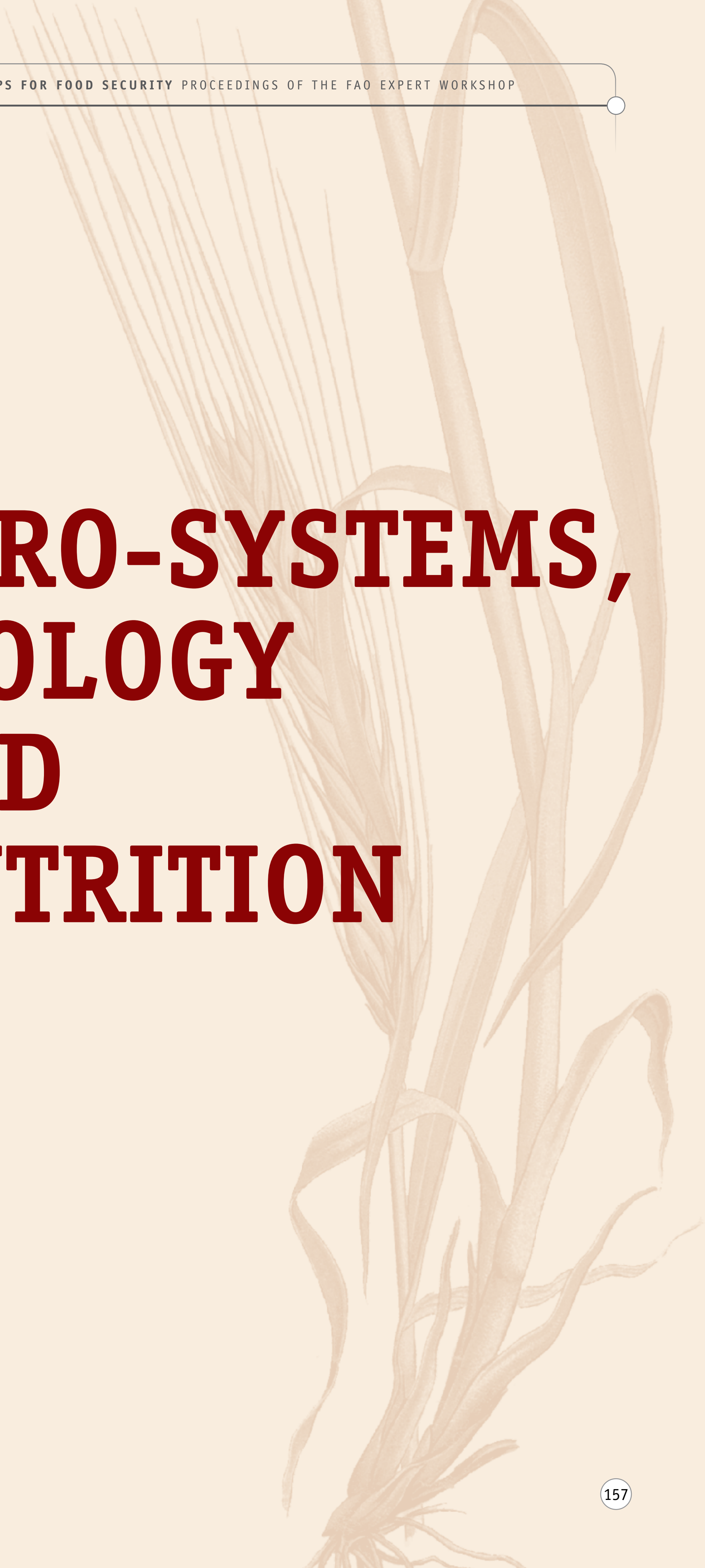
18 Are perennial crops more adapted to maintain long-term relationships with soils and, therefore, to sustainable production systems, soil restoration and conservation?

19 Perennial grain systems: a sustainable response to future food security challenges

20 Perennial grains: beyond bootlegging, feasibility and proof-of-concept



21 A new species of wheat that continues to grow after harvest



AGRO-SYSTEMS, ECOLOGY AND NUTRITION

12

FROM GENETICS AND BREEDING TO AGRONOMY TO ECOLOGY

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ABSTRACT

With annual grain monocultures, there is frequent clear-cutting of crop root systems; as a result, ecosystem processes below the soil surface cannot sustain themselves in a healthy state. Each year, we disrupt this rich ecological resource, which otherwise could have been contributing to high, stable grain yields. The science of agronomy arose out of the necessity to cope with such a compromised landscape and make up for lost ecological processes. But we are severely limited in what we can accomplish with the "software" we call agronomy as long as we are saddled with the deficiencies of the annual crop species that have always served as the "hardware". Once perennial grain-producing species become available, however, soil ecosystems can contribute much more to food production, while much of the landscape-repair work that we now call upon agronomy to provide will not be necessary. Perennial hardware will open up innumerable possibilities for bringing new ecological software into agriculture. An example is provided by the potential development of perennial sorghum for tropical regions. To date, breeding of winterhardy grain sorghum has occurred solely in North America. When those breeding populations begin to be evaluated in diverse tropical environments, selection for proper adaptation will be essential. Once that is accomplished, an interim objective may be to develop a “super-ratooning” sorghum. But in the longer term, a true perennial growth habit will make possible whole new farming systems that combine sorghum with perennial food legumes and other crops. We can make such systems productive and ecologically sound without resorting to many of the agronomic interventions that annual grain crops require.

Keywords: crop root system, soil ecosystem, grain sorghum, perennial food legumes, agriculture and ecology

INTRODUCTION

In recent years, the potential benefits of perenniality for agriculture have come to be widely recognized. For example, in 2009, a group of researchers assembled by the Royal Society in the United Kingdom endorsed the idea of perennial grains. They noted, “Perennial crops would store more carbon, maintain better soil and water quality and would be consistent with minimum till practice. These crops would also manage nutrients more conservatively than conventional annual crops, and they would have greater biomass and resource management capacity” (Royal Society, 2009).

In a further discussion of perennial grains’ potential, the National Research Council of the National Academy of Science in the United States went into more detail with the following statements (National Research Council, 2010):

- Perennial plants reduce erosion risks, sequester more carbon, and require less fuel, fertilizer, and pesticides to grow than their annual counterparts.
- Perennials have greater access to resources over a longer growing season.
- Perennials have greater ability to maintain the health and fertility of a landscape over longer periods of time.
- Recent advances in plant breeding... provide new opportunities for plant breeders to select for desired characteristics.

In a policy paper in the journal *Science*, twenty-eight United States and international scientists called on plant breeders and geneticists worldwide to launch an all-out effort to develop perennial grain crops. (Glover *et al.* 2010). Then in August 2013, perennial grain researchers from around the world met in Rome for their first global meeting: the FAO Expert Workshop on Perennial Crops for Food Security (this volume).

These and other recent events have been prompted by the recognition that substituting perennial cereals, grain legumes, and oilseeds for their annual counterparts would constitute a major change of crop “hardware”, enabling agriculture to support a vast new range of cropping-system “software.” The transformation will make possible food-producing ecosystems that function as well as healthy, fully intact natural ecosystems. Previous efforts to create such systems have been limited by our dependence on the problematic software of annual crops. Perennial grains will provide a solid foundation on which necessarily complex food-producing ecosystems can be built.

In doing this work, it will be essential to draw upon the extensive research that has been carried out by ecologists over more than a century. Embedded within wild ecosystems are answers needed in the design of current and future agricultural ecosystems. Processes that have run for many millions of years, some known and some yet to be discovered, are of paramount interest if we are to develop a sustainable, resilient agriculture. Moreover, successful use of the ecosystem as a conceptual tool can be expected to provide a bonus: greater social and economic justice for farmers, their communities, and the wider society.

Were we to stop with the development of perennial monocultures, we would miss the opportunity to realize the robust potential that biological diversity has to offer. To achieve sustainable nutrient management, stabilize or prevent insect and disease damage, optimize water use, take advantage of complementary growth periods, and ensure other important features of low-input farming will require information drawn from diverse natural ecosystems. Fortunately, the principles of ecosystem function derived from studies of grasslands, forests, and other non-cultivated landscapes are available for adoption (Jackson and Piper, 1989).

FROM AGRONOMY TO ECOLOGY

Starting with the invention of agriculture, annual grain monocultures have by definition required removal or disturbance of entire vegetative structures and communities. Ecosystem processes below the soil surface, opaque to the farmer, were crippled. This annual “clear-cutting” of the soil, which rendered a vast array of ecological processes unavailable to the food producer, has come at a heavy cost in degraded and eroded soil, water, and biodiversity.

The science of agronomy arose out of the necessity to mitigate and substitute for disrupted ecological processes in order to ensure a consistent food supply. Agronomy is a discipline forced to cope with a compromised landscape and make up for lost ecological processes through management. It can be viewed as a ten-thousand-year-old success story accomplished first by farmers alone, and in more recent times, by highly trained experts working with technologically astute farmers, backed up by fossil fuel subsidies and an industrialized, growth-oriented economy. But it is an unsustainable success story. With perennial cereals, grain legumes, and oilseeds, however, diverse ecological processes formerly denied to agricultural landscapes will have a chance to be reactivated and sustained over the long term.

The notion that native perennial ecosystems constitute the best model for agriculture is an acknowledgement and appreciation of the complexities and efficiencies in ecological relationships that have come about through community succession and natural selection operating on individual organisms over very long periods of time. As Jackson and Piper (1989) noted, "Nature is ill-defined, and natural ecosystems are dynamic. But the patterns and processes discernible in natural ecosystems still remain the most appropriate standard available to sustainable agriculture."

Deciding which aspects of non-agricultural ecosystems should inform the design of food-producing systems remains an open question. Denison *et al.* (2003) asked to what extent the outcomes of natural selection in native systems are congruent with the goals of agriculture:

> *We present two main hypotheses. Our first hypothesis is that natural selection had ample opportunity, before the wild ancestors of our crops were domesticated, to test alternative solutions to problems that limited individual fitness under preagricultural conditions... further improvement of these traits is likely to be difficult. Instead, opportunities for further genetic improvement of crop yield will mainly involve tradeoffs between plant adaptation to agricultural versus natural conditions, or between the competitiveness of individual plants and the collective performance of plant communities. Our second main hypothesis is that natural selection is the only reliable source of improvement (by any definition relevant to agriculture) in natural ecosystems that operate on a time scale longer than the lifetime of individual plants. Natural selection acts at the level of genes, individuals, and family groups, not communities and ecosystems. Therefore, our second main hypothesis is inconsistent with the suggestion that agricultural ecosystems whose structure is based on natural ecosystems will be consistently more efficient, sustainable and productive. Although this hypothesis rejects mindless mimicry, natural ecosystems, properly understood, are nonetheless a valuable source of ideas for agriculture.*

The perspective of Denison and colleagues has been valuable in motivating a deeper analysis and greater clarity in describing the value of natural ecosystems as conceptual tools for grain-producing agriculture. One crucial realization that has become clearer than ever is the extent to which their arguments rest implicitly on the assumptions that 1) progress in crop development equates to increased yield alone, and 2) the continued protection and facilitation of high yields with purchased inputs is both possible and desirable. An agriculture that requires minimal inputs and is resilient to changing environmental conditions is very different from the agriculture underlying Denison and colleagues' critique.

Natural selection acting on genes, individuals, and family groups can result in consistent properties that are manifest at the community and ecosystem level. This is different from selection happening at the community and ecosystem level. Consider, for example, the ability of members of the legume family to host nitrogen fixing bacteria in their roots. This mutualism is clearly the outcome of natural selection acting on legume plants and rhizobia bacteria; however, the entire ecosystem benefits from this mutualism as the N fixed by it is released for other organisms to appropriate upon legume senescence (Hooper and Dukes, 2004, DeHaan *et al.*

2010). The same is true for other nutrient acquisition strategies (e.g. Vance *et al.* 2003). In an agricultural system in which all nutrients are not provided in the form of mineral fertilizers, this type of community-level interaction becomes not only relevant but essential.

The life history strategy of perennials and their near-universal dominance of land ecosystems is another example of natural selection acting on the level of genes, individuals, and family groups and in turn predictably influencing community and ecosystem-level functions. While perenniality confers numerous advantages to individual plants existing in communities, it also prevents nutrient loss, soil erosion, weed invasion, and other ecological outcomes that benefit the ecosystem as a whole, whether native or agricultural (Picasso *et al.* 2008, Glover *et al.* 2010).

A contrasting example is provided by diversity. It is a reliable, naturally occurring characteristic of almost every native ecosystem, not because of natural selection, but more because of limits on the ability of natural selection to produce organisms that perform optimally under all environmental conditions in time and space. A diverse native plant community necessarily includes diversity of root architectures, leaf tissue chemistries, and other features. And when functioning together, these diverse plant attributes help prevent nutrient loss, explosion of insect herbivore populations, invasion of noxious weeds, and other ecological outcomes highly beneficial to agriculture (Ratnadass *et al.* 2012, Vandermeer, 2012). If insects and weeds are not to be controlled with pesticides, and prevention of soil erosion is to be independent of herbicide use, and if nutrient losses are not made up by application of non-renewable fertilizers, the ecological derivatives of diversity become even more important.

Perennialism per se, as suggested above, does constitute a significant mimicry of nature, and likely will bring with it a range of beneficial ecosystem functions. But it is important that we do not settle for the degree of improved ecosystem function that would be conferred, for example, by single-species stands of grain-producing perennial grasses. There is a reason why such an ecosystem is never seen in nature: it is not sustainable over time. Therefore, two strains of perennial grain research—breeding and ecology—must be carried out jointly.

BREEDING PERENNIAL GRAIN SORGHUM

Researchers aiming to develop food-producing ecosystems based on perennial grain crops face a chicken-and-egg situation: the perennial crop breeder would like to know more about the system into which the new species will be incorporated, but to study alternative systems, the ecologist needs the new crop species that do not yet exist. Nevertheless, much can be learned from experimental systems that incorporate the intermediate products of perennial grain breeding. Perennial sorghum provides one of many examples.

Sorghum is a tropically adapted genus. To survive and produce grain for more than one season in a temperate climate, a sorghum plant must not only produce rhizomes; those rhizomes must also be cold-tolerant enough to survive through the winter at between 2 and 20 cm below the soil surface and produce ramets that emerge in the spring. The Land Institute launched its research on perennial sorghum in the early 1980s by making hybrids between induced tetraploids of *Sorghum bicolor* and various rhizomatous, winterhardy accessions of the tetraploid grass *S. halepense*. Populations derived from those crosses, as well as from backcrosses to the *S. bicolor* parent, were evaluated in the field in Kansas, and the results were published by Piper and Kulakow (1994). They concluded that breeding of a perennial grain sorghum was feasible but would require repeated cycles of selection for perenniality and backcrossing to improve yield and other traits. Meanwhile, using RFLP mapping, Paterson *et al.* (1995) showed that genomic regions associated with rhizomatousness and perenniality in a diploid population derived from a *S. bicolor* x *S. propinquum* cross, when evaluated in a south Texas field environment, were scattered across at least nine of sorghum's ten linkage groups.

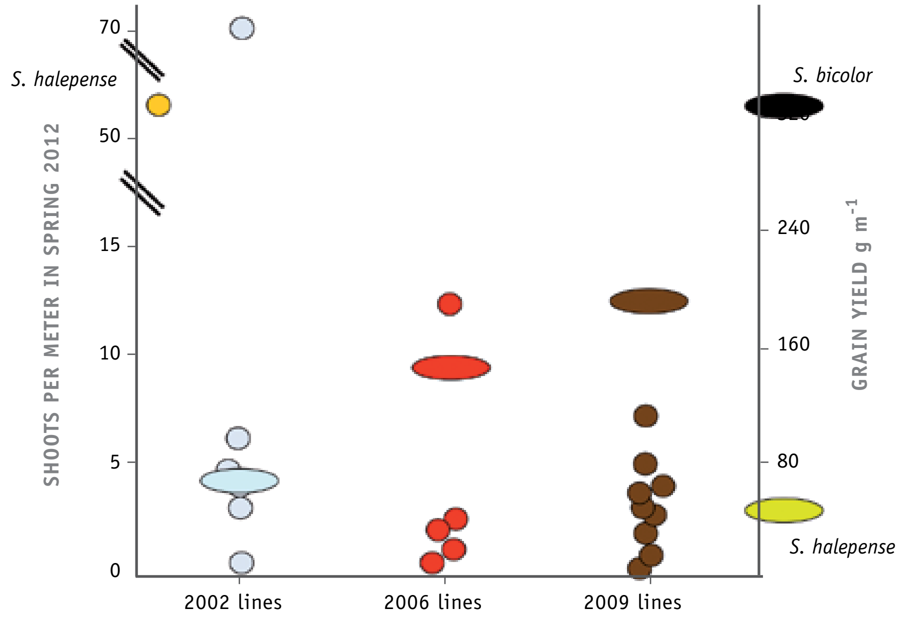
These basic studies were followed by the initiation, in 2001, of a perennial sorghum breeding programme at The Land Institute. The initial crosses were made between perennial selections from Land Institute populations and diploid grain sorghum inbred lines, to produce tetraploid segregating populations. Each year, we have selected perennial plants with improved cropping traits from annual x perennial or perennial x perennial populations and then backcrossed them to a range of annual lines, to capture more diversity from the *S. bicolor* gene pool. We have also introduced additional *S. halepense* accessions as parents.

In these populations, degree of perenniality is not associated with grain yield but is negatively correlated with kernel weight. Furthermore, most perennial selections tend to have traits that are generally undesirable in a crop plant: often excessive tillering and branching, non-synchronous flowering and maturity, excessive plant height, and small, hulled, brown kernels. Some of these associations, such as between rhizomatousness and aboveground tillering, may result from pleiotropy. Others, such as between winterhardiness and low kernel weight, may result from a low frequency of recombination between chromosomes of the two species in regions surrounding the genes required for perenniality. With Andrew Paterson at the University of Georgia, we have studies underway to elucidate more fully the basis for these negative associations (Paterson, this volume).

Despite the negative trait associations, selection and backcrossing have succeeded in increasing productivity while maintaining perenniality. In 2011-13, we ran a field trial comparing perennial families selected at three stages of The Land Institute's breeding program: 2002 (the original perennial parents), 2006, and 2009. Grain yield and kernel weight have increased with backcrossing and selection, but have not attained the levels of commercial grain sorghum (Figure 1). Perenniality is not as strong as that of *S. halepense*, but here, moderation is desirable; for a crop plant, the aggressive rhizome development displayed by the weedy parent would be an unnecessary and unproductive waste of resources. Among these selected lines, there was no correlation between degree of perenniality and grain yield; however, perenniality was associated with low weight of individual kernels (r=-0.46**).

FIGURE 1. NUMBER OF SHOOTS EMERGING IN SPRING PER METRE OF ROW IN SPRING, 2012, FOR 'GYPSUM 9' (*SORGHUM HALEPENSE*) AND 27 PERENNIAL SORGHUM FAMILIES SELECTED FROM THREE STAGES (2002, 2006, AND 2009) OF THE LAND INSTITUTE'S BREEDING PROGRAMME (CIRCLES AND LEFT Y-AXIS), TOGETHER WITH MEAN GRAIN YIELDS OF THE THREE GROUPS OF SELECTIONS (OVALS AND RIGHT Y-AXIS, WITH YIELDS OF GYPSUM 9 AND A COMMERCIAL GRAIN SORGHUM HYBRID, PHILLIPS 664, INDICATED ON THE AXIS.)

Yields are from first-year harvests of Salina, Kansas, retrospective trials in 2011 and 2012, with each year's trial in a randomized complete block design with four replicates. The experimental unit was a 3 m row.



Recently, we have selected lines potentially superior to those that were evaluated in the retrospective trial. These have come from crossing the *S. bicolor* inbred line BTx623 with plants from 'Gypsum 9', a *S. halepense* population collected near the town of Gypsum, Kansas in 2009. This cross was initiated to create populations for mapping loci associated with perenniality and other traits and monitoring recombination frequencies in interspecific hybrids of tetraploid sorghum (Paterson, this volume). But these populations have also become a valuable source of perennial breeding parents that have a more domesticated plant type and higher grain production. In these populations, associations between perenniality and deleterious wild traits appear to be weaker. Forty-nine partially winterhardy F_2-derived F_3 families derived from a BTx623 × 'Gypsum 9' cross and evaluated in 2011-2012 did not differ in mean height, flowering date, grain yield, and threshability from their winter-tender siblings (Table 1). However, strength of perenniality, expressed as the proportion of plants in a family that re-emerged in spring, had loose negative correlations with grain yield per plant and threshability.

TABLE 1. MEANS OF SIX TRAITS FOR GROUPS OF SORGHUM FAMILIES

49 families that had a percentage of spring emergence greater than zero, and 122 families with zero emergence, in a 2012-2013 field trial in Salina, Kansas. The families were F_3 progenies of 171 F_2 plants from a BTx623 (*Sorghum bicolor*) × Gypsum 9 (*S. halepense*) hybrid. They, together with parents, were evaluated in randomized, nonreplicated three metre rows with 1 metre between rows. Standard errors of means were based on variances of families within groups. Free-threshing index was the ratio of the weight of an approximately 5 g dehulled grain sample to the weight of the sample before dehulling; therefore, it has a possible range of 0 to 1. Correlations are rank correlations over 50 data pairs: 49 pairs of means of the families containing surviving plants, plus a single pair of means computed over all families that had zero survival.

		MEAN					
GROUP, BY SPRING EMERGENCE	No. OF FAMILIES IN GROUP	PROPORTION OF PLANTS EMERGED, SPRING	No. RAMETS, AUTUMN	PLANT HEIGHT	FLOWERING, DAYS AFTER 30 JULY	GRAIN YIELD PER PLANT, DEHULLED	FREE-THRESHING INDEX
No plants emerged	122	0	0.75	236	25.9	42	0.71
Some plants emerged	49	0.28	1.38 a	247	24.4	45	0.68
Correlation versus proportion of plants emerged			0.25	0.10	0.10	-0.34*	-0.31*

a Significantly different from the mean of the “no plants emerged” group (P<0.05)

* Significantly different from zero (P<0.05)

We still struggle to select for perenniality independently of other wild traits; however, among perennial BTx623 × ‘Gypsum 9’ selections, and among rhizomatous but cold-sensitive selections from other crosses, it has been possible to identify much more croplike lines that prove winterhardy in the warmer southeastern United States. These selections presumably would be perennial in the tropics as well. Therefore, we have been generating a second gene pool as a base population for breeding perennial sorghum for the tropics and semitropics. Breeding for tropical adaptation, which would start with crosses between temperately adapted perennial lines and tropically adapted annual lines, has not yet started. When it does, a medium-term outcome along the way to developing a rhizomatous perennial sorghum could be an improved rattooning sorghum that grows more vigorously and produces higher yields in the second (and possibly subsequent) season than do current annual cultivars (Paterson, this volume).

The current perennial sorghum gene pool bears only a loose resemblance to the annual grain crop. Dwarf plants are rare, and tillering is excessive. Ramet emergence, tillering, flowering, and maturity all are highly asynchronous, tending to extend over periods of weeks. Until these characteristics are adjusted through breeding, perennial sorghum in temperate regions will remain unsuitable for mechanized cultivation and harvest, whatever the degree of improvement in grain yield. However, in regions where hand harvesting and crop polycultures are the norm and where tall stover is harvested for fodder or other uses, tall plants and asynchronous maturity would not necessarily be obstacles to adoption. And perennial cultivars could have advantages beyond erosion prevention. In many parts of the tropics, one of the most dire problems facing sorghum farmers is poor stand establishment, thanks to adverse soil and moisture conditions. With perennial sorghum, there would be no need to achieve a good seedbed and precise seed placement under adverse conditions; furthermore, the shoots and roots of ramets emerging from deep rhizomes would be much more robust than those of seedlings.

But while presenting opportunities for adoption of perennial sorghum, tropical regions raise new challenges as well. Tropical environments are very diverse, and with seasonal variation in temperature and rainfall, most are not friendly to sorghum cultivation year-round. Research is needed to determine which environmental signals will be required to keep perennial sorghum plants alive but not actively growing during the off-season. In temperate zones, the onset of cold weather enforces several months of dormancy, after which new perennial plants emerge much earlier in the spring and grow more rapidly than do annual plants. But in the semiarid tropics, for example, would a perennial sorghum plant's rhizomes be able to survive deep in the soil through a long dry season and emerge with the return of rains? Answers to these and other questions have not yet been sought.

PROSPECTS FOR ECOLOGICALLY SOUND PRODUCTION

In trying to visualize agricultural ecosystems that include perennial sorghum and other perennial grains, the chicken-and-egg problem remains. How do we design ecologically sound production systems for new crops as long as the best of their germplasm is still half-wild? Will data gleaned from experiments using proto-perennial grains be applicable to cropping systems for fully domesticated cultivars once they are developed? We cannot know the answers to those questions in advance, but some insights could come from forging ahead with field research that employs today's crops-in-the-making.

Throughout tropical and semitropical regions, supplying grain crops with biologically fixed nitrogen, together with other nutrients, is an urgent need. If, as a first step toward a full-blown food-producing ecosystem, perennial sorghum is to be incorporated into a biculture, the obvious choice of partner species would be legumes—preferably perennial. The sorghum plant would derive the greatest fertility benefits from a non-grain legume that is included solely in a nitrogen-fixing role. But where increased food production per hectare and diet diversification are top priorities, an cereal-plus-grain legume intercrop is more appropriate. In Malawi, for example, Snapp *et al.* (2010 and this volume) have been investigating systems for growing pigeon pea (*Cajanus cajan*) as a perennial grain legume intercropped with annual maize (*Zea mays*). They have shown that "Semiperennial rotations provided twofold superior returns, whereas diversification of maize with annual legumes provided more modest returns" as compared with maize monocrops. These and other results provide an incentive, once perennial sorghum germplasm has been screened for adaptation to local conditions in areas where pigeon pea is well-adapted, to run small-scale pigeon pea intercrop experiments that include preliminary breeding lines of perennial or enhanced-rattooning grain sorghum. Such studies could proceed across a range of environments, in parallel with continuing breeding efforts until acceptable perennial sorghum cultivars are developed.

We are also investigating whether in this or other systems, perennial sorghum might supplement its nutrition through endophytic nitrogen fixation. It has been found, for example, that *S. halepense* harbours in its rhizomes nitrogen-fixing endophytic bacteria that also can make phosphorus more available and chelate iron (Weiman, 2012). As a genus, *Sorghum* also has a strong tendency to form associations with arbuscular mycorrhizal fungi and thereby increase its phosphorus uptake under certain conditions (Neumann and Eckhard, 2004); perennial sorghums for low-input systems should be evaluated for their ability to gain access to phosphorus and other nutrients through mycorrhizal associations.

With perennial sorghum, the development not only of farm-ready cultivars but also of ecologically-based cropping systems will probably be achieved first in the tropics. In many regions of Africa, Asia, or Latin America, a biculture of a perennial or rattooning cereal like sorghum with a perennial pulse could be a highly effective system for providing staple foods while reducing soil degradation, during the transition to more ecologically durable systems. Meanwhile, both resistance breeding and ecological research will be needed to address yield losses to pathogens, pests, and weeds, especially parasitic weeds like striga in the case of sorghum. More complex perennial polycultures involving a range of plant families will need to be evaluated, not only for productivity but for ecological functioning as well. For example, soil water and nutrient uptake efficiencies can be increased in polycropping arrangements where soil resources are partitioned in time or space.

Where agro-forestry is already being employed, integration with herbaceous perennial grain crops should be explored. Trees can improve crop microclimate conditions and provide nutrient inputs through biological N fixation and in some cases by bringing phosphorus and other rock-derived nutrients to the surface via roots from deep in the soil profile (Buresh, 1995). This could be particularly important in tropical regions with highly weathered, nutrient depleted topsoils. Some of this work can inform the eventual development of new cropping systems in the temperate zones, but much location-specific work will be required there as well.

The details will differ for other perennial crops, for other combinations of crops, and for every environment. But the ultimate goal remains constant: by adding perennial grain crops to the agricultural inventory, we can finally create opportunities for a deep synthesis of agriculture and ecology.

REFERENCES

Buresh, R.J. 1995. Nutrient cycling and nutrient supply in agroforestry systems. In: Dudal R, Roy RN (Eds.) *Integrated Plant Nutrition Systems*. FAO, Rome. pp. 155-164.

DeHaan, L.R., Weisberg, S., Tilman, D. & Fornara, D. 2010. Agricultural and biofuel implications of a species diversity experiment with native perennial grassland plants. *Agriculture, Ecosystems and Environment*. 137: 33-38.

Denison, R.F., Kiers, E.T. & West, S.A. 2003. Darwinian agriculture: when can humans find solutions beyond the reach of natural selection? *Quarterly Review of Biology*. 78: 145-168.

Glover, J.D., Reganold, J.P., Bell, L.W., Borevitz, J., Brummer, E.C., Buckler, E.S., Cox, C.M., Cox, T.S., Crews, T.E., Culman, S.W., DeHaan, L.R., Eriksson, D., Gill, B.S., Holland, J., Hu, F., Hulke, B.S., Ibrahim, A.M.H., Jackson, W., Jones, S.S., Murray, S.C., Paterson, A.H., Ploschuk, E., Sacks, E.J., Snapp, S., Tao, D., Van Tassel, D.L., Wade, L.J., Wyse, D.L. & Xu, Y. 2010. Increased food and ecosystem security via perennial grains. *Science*. 328: 1638-1639.

Hooper, D.U. & Dukes, J.S. 2004. Overyielding among plant functional groups in a long-term experiment. *Ecology Letters*. 7: 95-105.

Jackson, W. & Piper, J.K. 1989. The necessary marriage between ecology and agriculture. *Ecology*. 70: 1591-1593.

National Research Council. 2010. *Toward Sustainable Agricultural Systems in the 21st Century*. National Academies Press, Washington, DC.

Neumann, E. & Eckhard, G. 2004. Colonisation with the arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol. & Gerd.) enhanced phosphorus uptake from dry soil in *Sorghum bicolor* (L.) *Plant and Soil*. 261: 245–255.

Paterson, A.H., Schertz, K.F., Lin, Y.R., Liu, S.C. & Chang, Y.L. 1995. The weediness of wild plants: Molecular analysis of genes influencing dispersal and persistence of johnsongrass, *Sorghum halepense* (L.) Pers. *Proceedings of the National Academy of Sciences*. 92: 6127–6131.

Piper, J.K. & Kulakow, P.A. 1994. Seed yield and biomass allocation in *Sorghum bicolor* and F_1 and backcross generations of *S. bicolor* x *S. halepense* hybrids. *Canadian Journal of Botany*. 72: 468-474.

Ratnadass, A., Fernandes, P., Avelino, J. & Habib, R. 2012. Plant species diversity for sustainable management of crop pests and diseases in agroecosystems. *Agronomy for Sustainable Development*. 32: 273-303.

Royal Society. 2009. *Reaping the benefits: Science and the sustainable intensification of global agriculture*. The Royal Society, London.

Snapp, S.S., Blackie, M.J., Gilbert, R.A., Bezner-Kerr, R. & Kanyama-Phiri, G.Y. 2010. Biodiversity can support a greener revolution in Africa. *Proceedings of the National Academy of Sciences*. 107: 20840-20845.

Vance, C.P., Uhde-Stone, C. & Allan, D.L. 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytologist*. 157: 423-447.

Vandermeer, J.H. 2011. *The ecology of agroecosystems*. Jones and Bartlett Publishers, Sudbury, Mass.

Weiman, S. 2012. Symbiotic endophytes enable some grasses to fix N_2, outcompete natives. *Microbe*. 7: 445.

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ECONOMICS AND SYSTEM APPLICATIONS FOR PERENNIAL GRAIN CROPS IN DRYLAND FARMING SYSTEMS IN AUSTRALIA

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ABSTRACT

The development of perennial grains could provide a number of sustainability and farm management benefits for Australian dryland crop-livestock farming systems. Whole-farm bio-economic modelling has shown that perennial wheat would have greatest economic feasibility if it had dual-purpose attributes by providing additional forage post-harvest (during summer) and early in the winter growing season. This accrued from the ability to increase livestock numbers without a proportionate reduction in returns from grain production. Grain-only perennial wheat achieving similar prices would require yields of 60-100 percent of annual wheat to compare with current systems, while dual-purpose perennial wheat was still economically favourable with grain price AU$35/tonne less and grain yields 40 percent of annual wheat. In all cases perennial wheat would be most attractive on soils or situations where current annual cereal systems are most marginal. Cost-benefit analysis based on modelled increase in farm profit (AU$20/ha farm area), suggests that a 20 year investment in perennial wheat would result in a 10-fold return if it was adopted on 450 000 ha assuming 75 percent chance of success. While perennial wheat would have the largest impact in Australian farming systems, the development of perennial legumes for dual-purpose grain-grazing could also offer some potential. Several native Australian legumes have been assessed for their suitability and there are several short-lived perennial legumes that could have potential in such a system both in Australia and elsewhere (e.g. *Lablab purpureus*). Finally, perennial grain crop development should consider the range of farming systems where they might be used including facultative perennial systems, phase rotations (e.g. 2-4 year long rotations), companion or relay cropping (oversowing them with other crops/pastures) or polycultures involving a range of perennial species.

Keywords: dual-purpose, modelling, rotations, polyculture, companion cropping, cost-benefit

INTRODUCTION

Annual cereal crops, mainly wheat, in rotation with annual pastures have dominated grain production systems in Australia. This reliance on annual species has caused environmental problems such as dryland salinity, soil erosion and degradation, nutrient leaching and eutrophication. Reintroduction of productive and profitable perennial plants into agricultural landscapes to more closely mimic the original vegetation by increasing ground cover and annual water use can address many of these problems (Hatton and Nulsen, 1999). Farming systems incorporating agro-forestry and perennial forage plants are being utilised in many areas (Bell *et al.* 2013), but perennial grain crops could also provide a major opportunity to improve the sustainability of agricultural systems without the need to discontinue cropping activities (Glover *et al.* 2010; Bell *et al.* 2010b).

Perennial grain crops might be developed from either domestication of promising wild species or via hybridization of current annual crops with their perennial relatives (Cox *et al.* 2002). Both these avenues hold promise for developing perennial grain crops suitable for Australian conditions, but significant breeding effort would be required (Larkin, 2013). While breeding a genetically stable, productive and persistent perennial crop holds many challenges it is vitally important to consider how a perennial crop might be used in a farming system. This can provide insights that will guide the characteristics required in the crop and their relative importance for breeding efforts. While a number of sustainability benefits from perennial wheat are predicted and cost saving such as reduced tillage, fertiliser requirements and energy inputs are anticipated, the relative profitability of perennial grain crops compared with conventional annual systems needs to be analysed to justify investment in perennial crop development. This can also potentially expose the economic trade-offs between different attributes and help identify diverse crop ideotypes that might be valuable in different farming systems. This paper will provide a summary of some whole-of-system economic analysis conducted in Australia and consideration of diverse options for integrating perennial grain crops into farming systems. This provides some useful insights into priorities and strategies, and identifies opportunities for perennial grain crop development more widely. In particular, much attention has been applied to perennial wheat hybrids, yet there may be opportunities for complementary perennial grain legumes or other cereals either from direct domestication of native species or targeted breeding of other species.

PRELIMINARY ECONOMIC ANALYSIS OF PERENNIAL CEREAL CROPS IN AUSTRALIAN FARMING SYSTEMS

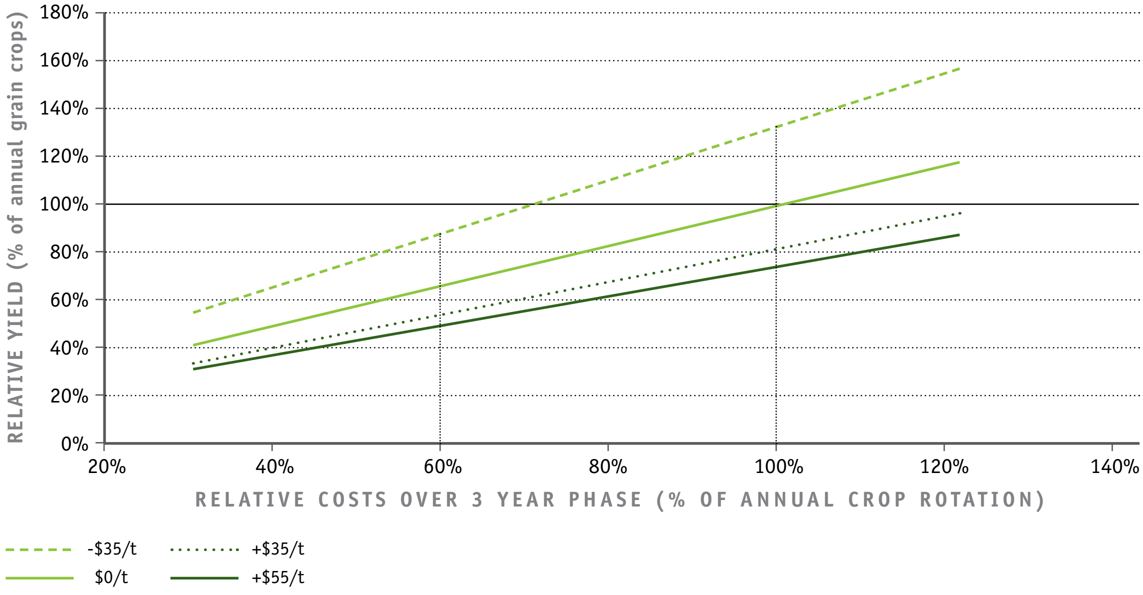
Grain-only production

Gross margins and whole-farm economics of a perennial cereal utilised for grain production were only compared with returns from conventional annual crop-based systems (based on a wheat-wheat-grain legume rotation). Yield, price and costs for the annual crop rotation were drawn from data for the medium rainfall regions of south-western Australia (350-500 mm mean annual rainfall) (more details are available in Bell *et al.* 2008). Because the income and costs for a perennial cereal system are uncertain, the sensitivity of break-even profitability was explored across a range of relative grain price, yield and variable production costs between the perennial and annual crop phases.

Figure 1 depicts the relationships between relative grain yield, growing costs and grain price on the relative profitability of a perennial crop compared with a typical annual crop rotation. This demonstrates the importance of the likely market for grain products on the cost-price requirements for a perennial grain crop. For example, if a grain-only perennial crop received a price premium or incentive payment of >US$35/tonne then it would achieve equal economic returns with grain yields 70-80 percent of annual crop yields at the same production cost per hectare. On the other hand, a lower quality grain product, similar to an animal feed grain receiving US$35 less per tonne than a milling grade product would require significantly higher grain yields than an annual grain crop system; an unlikely scenario for a perennial grain crop. One claim is that perennial grain crops will have lower production costs due to savings from less frequent sowing, herbicide applications and lower fertiliser replacement requirements (Crews 2005). Bell *et al.* (2008) estimate this could be 60 percent of an annual crop system. This would enable grain yields to be 50 percent and 65 percent of annual crops if a perennial cereal received a US$35/tonne price premium or the same price as an annual cereal crop, respectively. A perennial cereal receiving a lower price differential would require grain yields of >80 percent of an annual crop rotation to obtain a similar return.

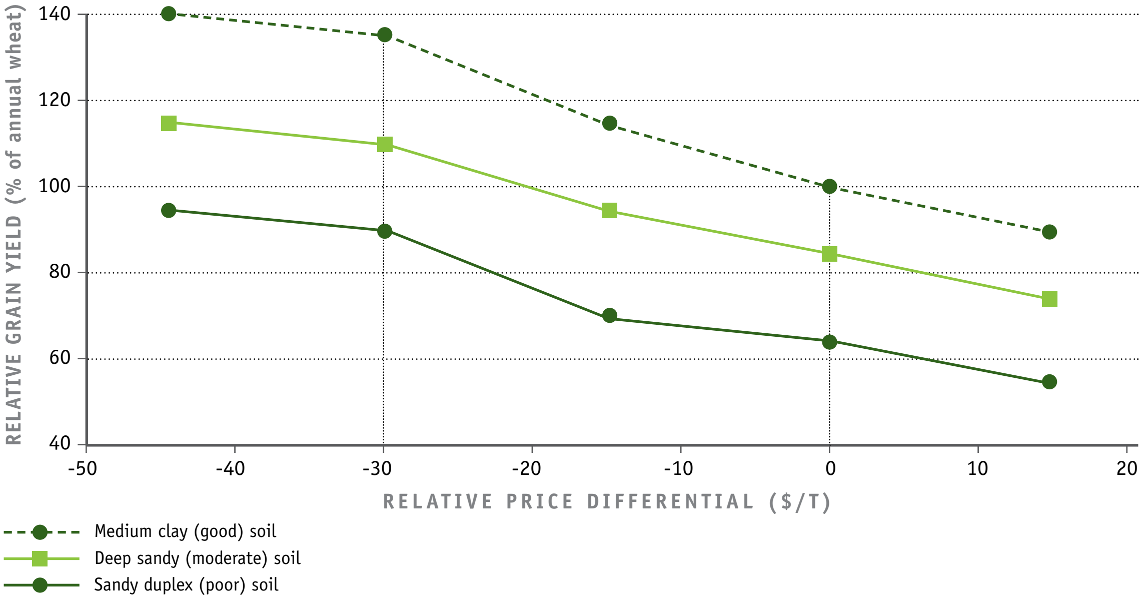
This analysis in Figure 1 was based on a 3-year phase of a perennial crop but the longevity of a perennial crop phase is also a factor that may impact on its relative profitability compared with annual crop systems. The yield required for a perennial crop to compare with an annual crop system declines with the duration of a perennial crop phase, as establishment costs are spread over more years. However, this reduction in grain yield required is small (<3 percent) once the duration of the perennial crop rotation is greater than three years because annual production costs (i.e. replacement fertiliser, harvesting costs) remain consistent. This suggests that unless perennial wheat yields are stable or increase with age of the stand, then there is little direct economic advantage in long-lived perennial crops. This is especially pertinent when considering potential trade-off that may exist between plant grain yield and longevity (see DeHaan *et al.* 2005).

FIGURE 1. GRAIN YIELD REQUIRED BY GRAIN-ONLY PERENNIAL CEREAL TO OBTAIN SIMILAR 3-YEAR GROSS MARGIN RETURNS TO AN ANNUAL CROP ROTATION (WHEAT-WHEAT-GRAIN LEGUME) ACROSS A RANGE OF RELATIVE GROWING COSTS AND GRAIN PRICE DIFFERENTIALS FOR PERENNIAL WHEAT COMPARED WITH THE ANNUAL CROPS



A perennial grain-only cereal crop that yielded 60 percent of an annual wheat crop with 60 percent of the variable costs but receiving US$35/tonne lower price was made available in a whole-farm bio-economic profit optimising model (MIDAS) (Morrison *et al.* 1986). The whole-farm model captures many of the biological and economic interactions that occur across a whole farm including variation in soil capabilities (denoted by different land management units with specific production and cost structures), rotational impacts and farm overheads (for more detail refer to Bell *et al.* 2008). Using the standard production/cost assumptions, a perennial grain crop producing only grain was not chosen on any land management units. The shadow or opportunity cost for a perennial cereal to be adopted on the farm was lowest on the less productive soil types (around US$25/ha), but was much higher on land management units where other annual crop and pasture systems were more profitable (US$40-100/ha). Subsidisation or other additional systems benefits would have to be larger than this to encourage adoption of a grain-only perennial cereal. Figure 2 demonstrates the relative grain yield and price required by a perennial cereal crop for it to be adopted across soil types differing in their productivity on a profit-maximising farm. This indicates that a grain-only perennial cereal is most likely to be adopted if it can be used on poorer or more marginal soil types for annual crop and pasture systems. On the most productive and profitable soil types grain returns similar to annual cereal crops would be required to displace current systems.

FIGURE 2. RELATIVE GRAIN YIELD REQUIRED FOR GRAIN-ONLY PERENNIAL CEREAL AT DIFFERENT PRICE DIFFERENTIALS COMPARED TO ANNUAL BREAD WHEAT TO BE PROFITABLY INCORPORATED ONTO DIFFERENT SOIL TYPES OF A DRYLAND FARM IN SOUTH-WESTERN AUSTRALIA



Dual-purpose graze and grain option

Because of the longer growing season, the potential to maintain green leaf for longer than an annual crop and respond to out-of-season rainfall, a perennial crop is likely to provide some additional forage compared with annual grain crops. This opportunity is likely to be similar, but perhaps larger than where long-season wheats are currently grazed during their vegetative growth period early in the growing season and allowed to regrow to produce grain later in the season (Bell *et al.* 2013). Whole-farm bio-economic modelling was necessary to determine if such a dual-purpose perennial crop may offer advantages to livestock production and whole-farm productivity over annual crops in mixed crop-livestock farming systems. This approach enables many of the complex interactions between crop and livestock enterprises, timing of livestock feed supply and the economics of the whole farm system to be analysed concurrently. In addition to standard production and price assumptions described previously, high quality green forage was made available for grazing early in the growing season following the break of season (i.e. start of the rain prior to sowing annual crops) and/or additional green forage was available after harvest in addition to the crop residue or stubble from the annual crops. No yield penalty from grazing was assumed.

Additional grazing obtained from a perennial cereal crop greatly improved its profitability and resulted in 20 percent of the profit-maximising farm plan being allocated to the perennial crop under standard assumptions. Again this was mainly on the soil types where other crop and pasture systems were least profitable even though lower production of grain and forage was assumed on these soils for the perennial cereal crop. Hence a perennial cereal crop was found to be a profitable addition to a mixed crop-livestock enterprise in southern Australia when it provided an additional 900 kg/ha post-harvest forage and 700 kg/ha early season forage, a yield 60 percent of annual wheat at a AU$35/tonne lower grain price and 60 percent of the production costs.

Modelling suggested that the dual-purpose perennial cereal crop could increase farm profit by 38 percent or AU$21 per farm hectare (Table 1), which equates to a net gain of AU$105/ha of perennial cereal sown (i.e. change in farm profit per unit area sown to the perennial cereal in the optimal scenario). This mainly came about through the ability to increase livestock numbers by providing forage at key times of the year. This also brought about structural changes in farm allocation between crops and pastures by enabling improved utilization of pastures by deferring the use of supplements, and an increase in pasture area on the farm to support this higher potential stocking rate. The dual-purpose crop also reduced the grazing of crop residues or stubbles which may also have other environmental and production benefits on other parts of the farm.

TABLE 1. FARM PROFITABILITY, ALLOCATION OF LAND TO CROP AND PASTURE, AND LIVESTOCK NUMBERS AND SUPPLEMENTATION UNDER AN OPTIMAL FARM PLAN WITH AND WITHOUT THE INTEGRATION OF A DUAL-PURPOSE PERENNIAL CEREAL

	WITHOUT GRAZING	WITH GRAZING	CHANGE (%)
FARM PROFIT (AU$/HA)	$55.6	$76.5	+ $21 (38%)
PERENNIAL CEREAL AREA (%)	0	20	+ 20
CROP AREA (%)	55%	45	- 10
PASTURE AREA (%)	45%	55	+ 10
STOCKING RATE (DSE/HA)	7.6	8.9	+ 1.3 (17%)
SUPP FEED (KG/DSE)	59.4	58.4	- 1.0

Adapted from Bell *et al.* (2008).

Due to uncertainty about the amount and timing of additional forage that might be provided by a dual-purpose perennial grain crop, a sensitivity analysis to these factors showed that a perennial grain crop providing even less additional forage could still be valuable and there is capacity to trade-off between forage and grain yield. Table 2 shows that forage provided early in the growing season before other feed sources are available is particularly valuable. Even small amounts of forage, as little as 175 kg/ha, provided at this time would increase farm profit and see 10 percent of farm allocated to perennial wheat. Providing forage after harvest was less valuable, but perennial wheat was still a profitable addition to the farm when only 500-1 000 kg of additional forage was provided after harvest only.

TABLE 2. SENSITIVITY OF AREA OF PERENNIAL CEREAL SELECTED (% OF FARM) IN THE OPTIMAL FARM PLAN TO THE TIMING AND AMOUNT OF FORAGE SUPPLIED FROM A DUAL-PURPOSE PERENNIAL CEREAL

EARLY GROWING SEASON		AFTER HARVEST ONLY		BOTH AFTER HARVEST AND EARLY SEASON	
Additional forage (kg/ha)	% perennial wheat	Additional forage (kg/ha)	% perennial wheat	Additional forage (kg/ha)	% perennial wheat
700	12	900	11	1600	20
525	10	675	13	1200	16
350	13	450	0	800	13
175	11	225	0	400	11

Adapted from Bell *et al.* (2008).

Table 3 also shows a further sensitivity analysis showing the area of the farm that would be sown to a perennial cereal under the optimal farm plan where lower levels of additional forage are supplied and lower relative grain yields were provided by a perennial cereal crop. This demonstrates that there is potential to trade-off between the grain yield and forage provided by a dual-purpose perennial grain crop. For example, if 800 kg/ha of additional forage was provided (350 kg/ha early in growing season and 450 kg/ha after harvest), perennial grain yields could be as low as 40 percent of annual wheat and still make a positive economic impact and would be included in an optimal farm plan on mixed crop-livestock farms. This shows that grain yields as low as 40 percent of an annual wheat might be feasible in a perennial cereal crop if it provides modest levels of additional green forage at key times of year. This also has significant implications for the attributes that might be targeted in developing perennial grain crops; revealing that forage production may be a vital attribute to consider and that lower grain yields could be profitable if additional forage for livestock at key times could be obtained from a perennial grain crop.

TABLE 3. SENSITIVITY ANALYSIS TO LOWER AMOUNTS OF ADDITIONAL FORAGE SUPPLY AND LOWER RELATIVE PERENNIAL CEREAL GRAIN YIELD ON THE AREA OF PERENNIAL WHEAT (% OF FARM AREA) UNDER THE OPTIMAL FARM PLAN

Standard assumptions were a relative grain yield of 60 percent and 1600 kg/ha of additional forage supplied.

FORAGE SUPPLIED AFTER HARVEST AND EARLY GROWING SEASON (kg/ha)	RELATIVE PERENNIAL WHEAT GRAIN YIELD		
	60%	50%	40%
1600	20%	19%	14%
1200	16%	16%	14%
800	13%	13%	12%
400	11%	0%	0%

Source: Bell *et al.* 2008.

Residual indirect production and environmental implications

It is important to point out that the above economic analyses considered only the direct production implications of a perennial cereal in a conventional dryland farming systems. This did not consider the implication of other technologies such as perennial pastures or dual-purpose annual crops which might diminish the relative benefit obtained from forage provided by perennial wheat. There are also several other indirect and sustainability factors are also important. No value was attributed to environmental benefits that a perennial crop might provide, whether this is an impact on the long-term condition of the land where a perennial crop is grown (e.g. soil carbon, soil erosion) or off-site external impacts (e.g. water eutrophication, sediment flow, greenhouse gas emissions). While the maintenance of land condition may directly influence a farmer's intention to adopt a perennial crop, without policy or economic incentives it is unlikely the off-site externalities would play a major part of farm decision making. However, a perennial crop may also offer several whole-farm management benefits that might prove attractive to farmers (Bell *et al.* 2010b). Firstly, because seeding frequency would be reduced, farmers could adjust or reduce their capital investment in seeding and spraying equipment, or alternatively farm a larger area without the need for more machinery. This would also be beneficial by reducing labour requirements at peak times of the year such as sowing. Secondly, the possibility of utilising perennial wheat for either grain or grazing purposes would enable greater enterprise flexibility where producers could delay their decision beyond the time of planting in response to climatic and economic conditions. Because of lower external inputs such as crop establishment costs, fertilizer and herbicides (as discussed previously), the risk exposure to climatic and market fluctuations is also reduced.

INTEGRATING PERENNIAL CEREALS INTO FARMING SYSTEMS

In addition to the economic and agronomic characteristics outlined above, perennial cereals could be utilised in a range of different ways in a farming system which may require different attributes (Bell *et al.* 2010b). One of the most important of these is the persistence or stand longevity of a perennial cereal which will influence the permanency, or length of time the perennial crop remains in the system. Hence, depending on the capacity of a perennial crop to persist or maintain productivity in different agro-climatic zones or conditions, different uses of perennial wheat may be appropriate. Below some likely systems are discussed where a perennial cereal could conceivable fit into a farming system

Perennial grain polycultures

In North America, the ultimate system for perennial grain production proposed by some would involve a permanent ‘polyculture’ mixture including warm- and cool-season perennial grasses, perennial legumes and composites that mimic their natural prairie systems (Piper, 1998)(Figure 3a). Perennial mixtures are thought to be more sustainable than pure stands, and have been shown experimentally to produce more grain yield and biomass from mixtures compared with monocultures of each species (Piper, 1998). This is because there were synergies between the functional groups, such as legumes compensating for low N supply. However, realizing successful perennial crop polycultures would require species that complement one another spatially, seasonally or in nutrient requirements, so that (a) land, labour or resources are used more efficiently; (b) yield is increased; (c) losses to insects, diseases, and weeds are reduced; or (d) yield variation is reduced (Piper, 1998). The development of such a system is an ambitious undertaking and its complexity would bring challenges. Water limitations during the wheat growing season in many Australian cropping systems, may impose significant competition between components of a mixture, especially among competitive perennial species. Many Australian environments also have short growing seasons and hence it would be difficult to time the growth, development and harvest of diverse grain crops into such a short period.

Companion or relay cropping

It is likely that in Australian systems, much simpler mixtures of perennial cereals would be more suitable. Companion or relay of other crops (e.g. grain legumes or cereals) into existing perennial crop stands may provide several of the benefits of mixtures (e.g. N inputs, enable competition to be managed and increased system productivity). Inputs of nitrogen might be obtained by growing an annual pasture legume (e.g. medic or clover) under a perennial cereal to provide a cheap source of N, as well as to compliment grazing from a perennial cereal (Figure 3b). Similar systems involving perennial pasture grasses in mixtures with annual legumes already exist in many environments (see Hayes *et al.* 2013, this conference). In more arid environments, where lower densities of perennial plants persist, companion cropping during periods favourable for growth could be a good strategy for increasing productivity (Figure 3c). In higher rainfall regions of southern Australia, a companion crop could be grown at the same time as the perennial cereal during the moist winter-growing period without excessive competition for water. For example, in some regions in the uniform rainfall zone of southern NSW, annual cereal grain crops are sown into permanent perennial grass pastures (pasture cropping) which is being found to increase water utilization, and enable fertilizer inputs that benefit the productivity of the perennial system (Miller and Badgery, 2009). Where longer growing seasons or summer rainfall is higher, relay systems might enable a crop or forage to be sown or regenerate after the harvest of the perennial wheat. Annual decisions on whether or not to companion or relay crop the perennial wheat could be made tactically based on seasonal prospects, the requirements for nitrogen inputs, disease pressures and perennial crop densities.

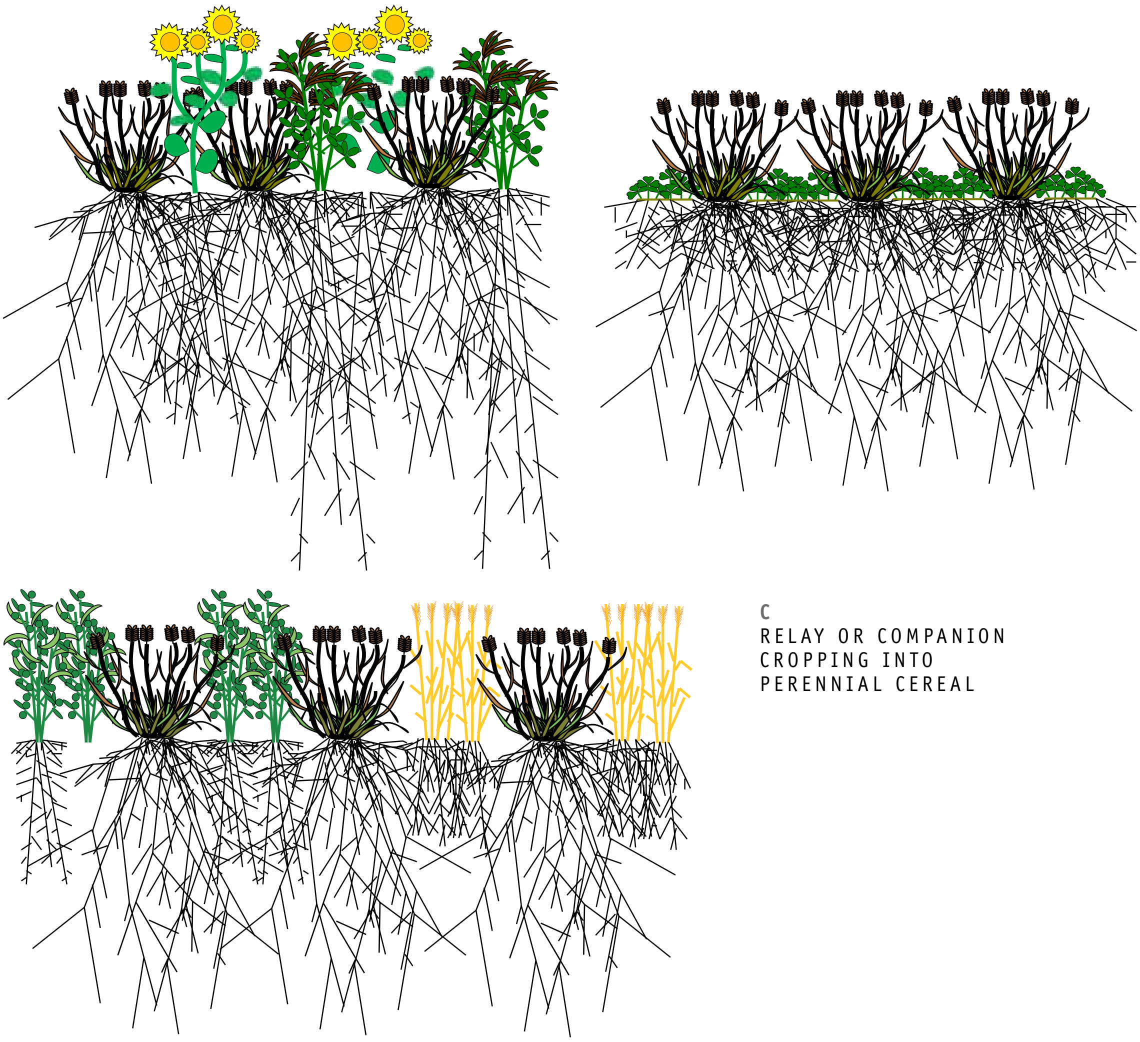
Phase rotations

A perennial crop might be used in a similar way to perennial pasture legumes and some grasses (e.g. alfalfa) as a phase of 2-4 years followed by a phase of annual crops or pastures (Figure 4). In this system, a perennial crop does not need to be long-lived and the perennial crop could be removed once plant populations or productivity decline. Alfalfa and other perennial pastures used in this way provide hydrological benefits by depleting subsoil water content and then allowing this dry soil buffer to refill during subsequent years of annual crops or pastures (Ridley *et al.* 2001; Ward 2006). The annual crop and pasture phase would also enable soil nutrient reserves to be replenished, provide disease break benefits should soil borne or foliar diseases build up and enable weed control options to be diversified.

Such a system is also self-regulating. In low rainfall environments, the length of the perennial phase would be shorter because subsoil water reserves are depleted and productivity of perennials decline more quickly, but in these environments drainage events below the annual crop root zone are less frequent, and longer phases of annual crops can be accommodated before dry subsoils are refilled (Ward, 2006).

FIGURE 3. DEPICTIONS OF ALTERNATIVE FARMING SYSTEMS INVOLVING PERMANENT PERENNIAL CEREAL CROPS

(a) polyculture mixture with perennial legume and composite as proposed by Piper (1998), (b) annual legume understory to provide N inputs and minimal competition for water during the growing season, (c) relay or companion cropping of annual grain legumes or cereals.



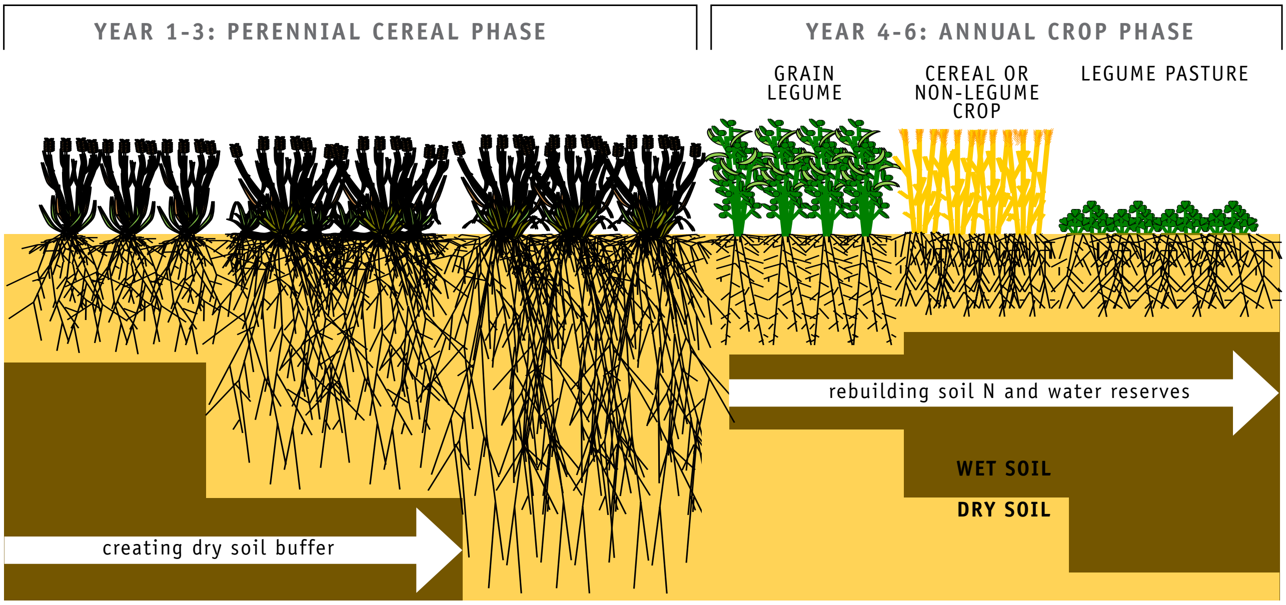
A
PERMANENT PERENNIAL GRAIN CROP POLYCULTURE

B
PERENNIAL CEREAL-ANNUAL LEGUME MIXTURE

C
RELAY OR COMPANION CROPPING INTO PERENNIAL CEREAL

On the other hand, like perennial pastures, perennial cereal persistence may be better in higher rainfall zones, and hence, allow longer rotations where a greater proportion of perennial is needed in crop rotations to reduce the higher drainage and runoff rates in these environments (Ward, 2006). Perennial wheat suitable for these phase rotation systems would need to produce grain in the first year, and be cheap and reliable to establish and remove.

FIGURE 4. DEPICTION OF A PHASE ROTATION INVOLVING A THREE YEAR PHASE OF A PERENNIAL CEREAL CROP WHERE SUBSOIL WATER AND MINERAL N IS DEPLETED FOLLOWED BY THREE YEARS OF ANNUAL CROPS AND PASTURES WHERE SOIL NUTRIENTS AND SUBSOIL WATER RESERVES ARE REPLENISHED



Facultative perennial crop systems

Even in situations where a perennial cereal has uncertain year-to-year persistence (e.g. low rainfall environments) there may still be a fit and advantages in farming systems. Such a perennial wheat genotype would require cost and ease of establishment and management, and grain yields similar to annual wheat and, in most years, would be analogous to using a long-season dual-purpose wheat. However, in favourable years when the perennial wheat was able to persist adequately, this may allow an opportunistic crop or may be used as a pasture in the subsequent year. By reducing the requirement for longevity and/or allocation of resources to survival strategies, higher grain yields might also be achievable more immediately.

PROSPECTS FOR OTHER DUAL-PURPOSE PERENNIAL GRAINS

While perennial cereals, based on wheat hybrids, would have the largest market opportunity in Australia, a range of other perennial cereals and non-cereal grain crops might have significant value in Australian farming systems. This is particularly the case if perennial crops have greatest opportunities on marginal or challenging soils to provide dual-purpose grazing and grain production opportunities.

Perennial grain legumes

The development of perennial legumes for dual-purpose grain-grazing could also offer some potential by providing high quality forage, in particular protein, for livestock at key times, provide inputs of N and disease management options in rotations, as well as potentially fitting on soil types where few grain legumes are well adapted. A perennial grain legume providing its own N needs would also reduce the need for the perennial crop to be grown in a mixture with other species, hence providing easier management of a monoculture.

A significant effort has been continuing in Australia to investigate the potential for a range of exotic and native perennial legumes as forage species but little attention has been applied to perennial grain legume options (Li *et al.* 2008). The native Australian legumes examined in this work were found to occur in areas with arid climates, and infertile and poor soils (Bennett *et al.* 2010; Pang *et al.* 2010) and hence were thought to be a good place to look for potential new grain crops adapted low-input agricultural systems in harsh growing conditions where other crop species may not be well suited. Australia's native legume flora is also largely unexplored for their potential as grain crops.

Examination of the natural distribution among a range of native legume genera found in semi-arid and arid regions of temperate Australia, together with information on traits linked to agronomic success as grain crops (i.e. harvestability, grain qualities and fecundity) revealed several species considered worthy of further evaluation for their grain production potential (Bell *et al.* 2010a). A range of these species have been grown under controlled conditions to compare their growth and reproductive traits, seed yield and composition with commercial annual grain legumes (Table 2; Bell *et al.* 2012). Seed yields of seven native perennial legumes were >40 percent of chickpea (*Cicer arietinum*), with grain protein, fat and fibre similar to the commercial grain legumes in the range desirable in food and feed industries. In several species the reproductive allocation were also similar to the annual commercial grain legume cultivars and much higher than might be expected from perennial species. These results are quite exciting for several reasons. Firstly, growing conditions were favourable to the annual species (well watered and fertilised in greenhouse), while under lower fertility and moisture limited conditions the relative productivity of the native perennials would be expected to be improved. Secondly, these results were based on only one accession of each taxon; undoubtedly there is substantial capacity to explore germplasm with greater productivity, larger seed size and phenological development. These species are also those that have been identified as having potential as forage plants and hence could have potential as dual-purpose options.

TABLE 4. SEED YIELD, HARVEST INDEX, SEED SIZE, CRUDE PROTEIN (CP) AND FAT CONTENT OF SEVEN UNDOMESTICATED AUSTRALIAN NATIVE LEGUMES COMPARED WITH TWO COMMERCIAL ANNUAL GRAIN LEGUMES (CHICKPEA AND FIELD PEA; HIGHLIGHTED IN GREY) WHEN GROWN IN A GREENHOUSE UNDER THE SAME CONDITIONS

SPECIES	SEED YIELD (g plant^{-1})	HI (g g^{-1})	SEED MASS (mg seed^{-1})	CP CONTENT (%)	FAT CONTENT (%)
Pisum sativa	9.9	0.50	258.9	26.3	1.2
Glycine species	4.8	0.54	11.2	32.2	5.2
*Cicer arietnum**	4.6	0.60	188.7	22.9	4.5
Lotus cruentus	3.4	0.65	1.5	32.0	5.9
Cullen tenax	2.8	0.30	5.2	32.1	11.3
Glycine canescens	2.7	0.35	16.9	34.0	6.2
Swainsona kingii	2.2	0.47	2.7	34.3	2.5
Cullen cinereum	2.1	0.30	5.2	36.2	11.8
Swainsona colutoides	2.0	0.21	3.1	27.5	2.1

* actual species is unknown

Adapted from Bell *et al.* (2012)

While this analysis examined potential in Australia's native herbaceous legumes adapted to temperate environments, there is also a range of tropically adapted perennial legumes that might have potential. For example, Australia possesses a large diversity of perennial legumes in the genera *Glycine*, *Crotalaria*, *Canavalia* and *Vigna* all which have close relatives which are grown as annual grain legumes (e.g. *Glycine max* – soybean, *Crotalaria juncea* – sunn hemp, *Vigna radiata* – mungbean) (Bell *et al.* 2010a). These perennial relatives of grain legume crops could provide a useful source of perennial germplasm adapted to arid conditions and infertile soils. Several tropical species such as lablab (*Lablab purpureus*) and pigeon pea (*Cajanus cajan*) are already used as annual dual-purpose crops in some countries (particularly in smallholder settings), but have germplasm that are short-lived perennials.

Other perennial cereals

A diverse range of other perennial cereal crops could have advantages over wheat in some situations. For example, perennial triticale could be produced from hybrids between *Triticum* species and *Secale montanum* with advantages over wheat due to its greater tolerance of acid soils (and high aluminium levels), low nutrient availability, drought and temperature stress (Jessop, 1996). Annual triticale is currently grown in Australia where wheat performance is reduced by these stresses and it is also widely used as a dual-purpose graze and grain crop. Hybridisation of *Triticum* with *S. montanum* should also be easier than with *S. cereale* (used to develop existing triticale), because the former is thought to be more closely related to wheat (Appels, 1982). Perennial grain rye using *S. montanum* has also been the target of some efforts internationally, and could improve the rooting depth, drought and heat tolerance of rye, but past efforts have encountered problems maintaining both perenniality and fertility (Reimann-Philipp, 1995). In Australia, *S. montanum* has been breed as a forage grass and hence adapted and agronomically suitable germplasm is likely to be available and may provide a useful starting point for any efforts to develop either a perennial rye or triticale (Oram, 1996). However, the increases in grain size above the commercial *S. montanum* forage variety would be required to produce a useful grain product and to increase grain yields (Hayes *et al.* 2012).

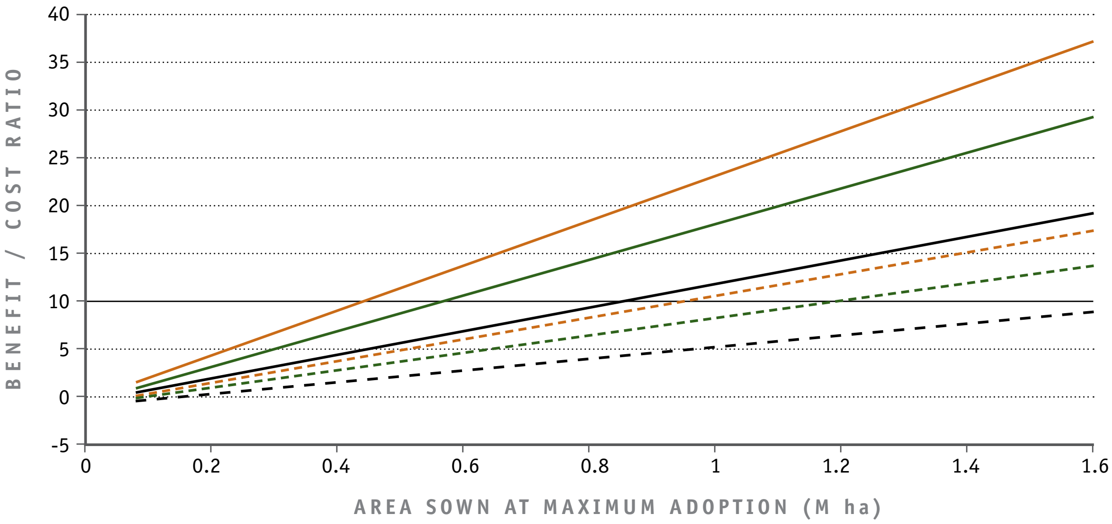
In addition, we should not discount direct domestication of already adapted native Australian grasses such as *Microleana stipoides* (Davies *et al.* 2005). This grass is an important forage species and has been shown to have many attributes suitable as a dual-purpose grain and graze crop. Warm season perennial cereals, such as sorghum and pearl millet, may also be better suited in Australia's northern grain growing zone where rainfall is summer dominant. In these environments, commercial sorghum crops regularly ratoon after harvest and sometimes survive for more than one year, unless they encounter severe frost. Hence, breeding a perennial sorghum suited to subtropical farming systems may involve selection from within the range of pre-breeding material already available and hence avoid challenges with wide hybridization.

VALUE PROPOSITION FOR INVESTMENT IN PERENNIAL CROP DEVELOPMENT

Despite the significant opportunities and benefits that development of a perennial grain crop could provide it is important to consider and establish the value proposition for investment in their development. That is, would breeding a perennial grain crop pay off economically? Based on the economic outcomes predicted from a dual-purpose perennial cereal in the whole-farm modelling described previously (i.e. AU$20/farm ha (as shown in Table 1) and AU$10/ farm ha (assuming smaller areas are adopted on farms; e.g. Table 3), Figure 3 shows the benefit-cost ratio (i.e. calculated cumulative economic return over 20 years divided by the cost of development, with a discount rate of 5 percent) across a range of scales of adoption and assuming investments of AU$1 million per year over 10, 15 and 20 years. This demonstrates that the likely scale of adoption is a key factor influencing the likely return on investment in a perennial crop. Successful peak adoption on 0.4-0.5 million ha would achieve a 10:1 minimum benefit/cost on a AU$20 million investment over 20 years with 75 percent probability of success. Lower probabilities of success even over shorter time-frames and/or lower returns per farm hectare challenge the capacity for a perennial cereal to produce such high returns on investment unless it was suitable for a large proportion of Australia's cropping zone.

FIGURE 5. RELATIONSHIP BETWEEN AREA ADOPTED AND BENEFIT/COST RATIO FOR AN INVESTMENT OF AU$1 M/YEAR IN THE DEVELOPMENT OF A PERENNIAL CROP ASSUMING 25 PERCENT LIKELIHOOD OF SUCCESS AFTER 10 YEARS (BLACK), 50 PERCENT LIKELIHOOD OF SUCCESS AFTER 15 YEARS (GREEN) AND 75 PERCENT LIKELIHOOD OF SUCCESS AFTER 20 YEARS (RED) ASSUMING A NET ECONOMIC ADVANTAGE OF AU$20/FARM HA (SOLID LINES) AND AU$10/FARM HA (DOTTED LINES)

For further details on assumptions refer to Bell *et al.* (2008).



CONCLUSION

This paper points out that it is important to consider the farming systems context into which a perennial grain crop might be introduced. This can help guide those qualities and attributes that might be most desirable and lead to greatest adoption and economic returns. In an Australian context is seems that a perennial grain crops with dual-purpose attributes providing grazing for livestock as well as grain yield and is adapted to the less productive parts of the landscape are likely to be the most advantageous in Australian farming systems. This can also offset initially lower grain yield and quality of a newly developed perennial cereal and provides an opportunity for a transitional genotype that might be developed based on forage grass that provides opportunistic grain production. While most of efforts so far have focussed mainly on wheat, there may actually be other perennial grain options which are easier to realize and could meet these requirements more easily (e.g. sorghum, triticale, perennial lablab or domestication of a native grass or legume). Similarly, a diverse range of farming systems could be developed in which a perennial crop might be used and wider consideration of these options should be taken in future breeding efforts.

REFERENCES

Appels, R. 1982. The molecular cytology of wheat-rye hybrids. *International Review of Cytology*. 80: 93-132.

Bell, L.W., Bennett, R.G., Ryan, M.H. & Clarke, H.J. 2010a. The potential of herbaceous native Australian legumes as grain crops: a review. *Renewable Agriculture and Food Systems*. 26: 72-91.

Bell, L.W., Byrne, F., Ewing, M.A. & Wade, L.J. 2008. A preliminary whole-farm economic analysis of perennial wheat in an Australian dryland farming system. *Agricultural Systems*. 96: 166-174.

Bell, L.W., Ryan, M.H., Bennett, R.G., Collins, M.T. & Clarke, H.J. 2012. Growth, yield and seed composition of native Australian legumes with potential as grain crops. *Journal of the Science of Food and Agriculture*. 92: 1354-1361.

Bell, L.W., Wade, L.J., Ewing, M.A. 2010b. Perennial wheat: environmental and agronomic prospects for its development in Australia. *Crop and Pasture Science*. 61: 679-690

Bell, L.W., Kirkegaard, J.A. & Moore, A.D. 2013. Evolving crop-livestock integration systems that improve farm productivity and environmental performance in Australia. *European Journal of Agronomy*. in press, (Available at www.sciencedirect.com/science/article/pii/S1161030113000543).

Bennett, R.G., Ryan, M.H., Colmer, T.D. & Real, D. 2010. Prioritisation of novel pasture species for use in water-limited agriculture: a case study of *Cullen* in the Western Australian wheatbelt. *Genetic Resources and Crop Evolution*. 58: 83-100.

Cox, T.S., Bender, M., Picone, C., Van Tassel, D.L., Holland, J.B., Brummer, E.C., Zoeller, B.E., Paterson, A.H. & Jackson, W. 2002. Breeding perennial grain crops. *Critical Reviews in Plant Sciences*. 21: 59-91.

Crews, T.E. 2005. Perennial crops and endogenous nutrient supplies. *Renewable Agriculture and Food Systems*. 20: 25–37.

Davies, C.L., Waugh, D.L. & Lefroy, E.C. 2005. Variation in seed yield and its components in the Australian native grass *Microlaena stipoides* as a guide to its potential as a perennial grain crop. *Australian Journal of Agricultural Research*. 56: 309-316.

DeHaan, L.R., Van Tassel, D.L. & Cox, T.S. 2005. Perennial grain crops: A synthesis of ecology and plant breeding. *Renewable Agriculture and Food Systems*. 20: 5–14.

Glover, J.D., Reganold, J.P., Bell, L.W., Borevitz, J., Brummer, E.C., Buckler, E.S., Cox, C.M., Cox, T.S., Crews, T.E., Culman, S.W., DeHaan, L.R., Eriksson, D., Gill, B.S., Holland, J., Hu, F., Hulke, F.S., Ibrahim, A.M.H., Jackson, W., Jones, S.S., Murray, S.C., Paterson, A.H., Ploschuk, E., Sacks, E.J., Snapp, S., Tao, D., Van Tassel, D.L., Wade, L.J., Wyse, D.L. & Xu, Y. 2010. Increasing food and ecosystem security through perennial grain breeding. *Science*. 328: 1638-1639

Hatton, T.J. & Nulsen, R.A. 1999. Towards achieving functional ecosystem mimicry with respect to water cycling in southern Australian agriculture. *Agroforestry Systems*. 45: 203-214.

Hayes, R.C., Newell, M.T., DeHaan, L.R., Murphy, K.M., Crane, S., Norton, M.R., Wade, L.J., Newberry, M., Fahim, M., Jones, S.S., Cox, T.S. & Larkin, P.J. 2012. Perennial cereal crops: An initial evaluation of wheat derivatives. *Field Crops Research*. 133: 68-89.

Hayes, R.C., Newell, M.T. & Norton, M.R. 2013. Agronomic management of perennial wheat derivatives: using case studies from Australia to identify challenges. In: C. Batello, L. Wade, S. Cox, N. Pogna, A. Bozzini, J. Choptiany (eds) *Proceedings of the FAO Expert Workshop on Perennial Crops for Food Security,* 28-30 August 2013, Rome, Italy, p 339-361.

Jessop, R.S. 1996. Stress tolerance in new triticales compared to other cereals. In: H. Guedes-Pinto, N. Darvey, V.P. Carnide (eds), *Triticale: today and tomorrow*, Kluwer Academic Publishers, Dordrecht, The Netherlands, p 419-427.

Larkin, P.J. & Newell, M.T. 2014. Perennial wheat breeding: current germplasm, and a way forward for breeding and global cooperation. In: C. Batello, L. Wade, S. Cox, N. Pogna, A. Bozzini, J. Choptiany (eds), *Proceedings of the FAO Expert Workshop on Perennial Crops for Food Security*, 28-30 August 2013, Rome, Italy, p 39-53.

Li, G.D., Lodge, G.M., Moore, G.A., Craig, A.D., Dear, B.S., Boschma, S.P., Albertsen, T.O., Miller, S.M., Harden, S., Hayes, R.C., Hughes, S.J., Snowball, R., Smith, A.B. & Cullis, B.C. 2008. Evaluation of perennial pasture legumes and herbs to identify species with high herbage production and persistence in mixed farming zones in southern Australia. *Australian Journal of Experimental Agriculture.* 48: 449–466.

Millar, G.D. & Badgery, W.B. 2009. Pasture cropping: a new approach to integrate crops and livestock farming systems. *Animal Production Science.* 49: 777-787.

Morrison, D.A., Kingwell, R.S., Pannell, D.J. & Ewing, M.A. 1986. A mathematical programming model of a crop livestock farm system. *Agricultural Systems.* 20: 243–268.

Pang, J., Ryan, M.H., Tibbett, M., Cawthray, G.K., Siddique, K.H.M. & Bolland, M. 2010. Variation in morphological and physiological parameters in herbaceous perennial legumes in response to phosphorus supply. *Plant and Soil.* 331: 241-255.

Piper, J.K. 1998. Growth and seed yield of three perennial grains within monocultures and mixed stands. *Agriculture, Ecosystems and Environment.* 68: 1-11.

Oram, R.N. 1996. *Secale montanum*: a wider role in Australasia? *New Zealand Journal of Agricultural Research.* 39: 629–633.

Reimann-Philipp, R. 1995. Breeding perennial rye. *Plant Breeding Reviews.* 13: 265–292.

Ridley, A.M., Christy, B., Dunin, F.X., Haines, P.J., Wilson, K.F. & Ellington, A. 2001. Lucerne in crop rotations on the Riverine Plains. I. The soil water balance. *Australian Journal of Agricultural Research.* 52: 263-277.

Ward, P.R. 2006. Predicting the impact of perennial phases on average leakage from farming systems in south-western Australia. *Australian Journal of Agricultural Research.* 57: 269-280.

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FROM FIELD TO TABLE: PERSPECTIVES AND POTENTIAL FOR FRUIT DOMESTICATION

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ABSTRACT

Domestication, the evolution of species in response to human selection, is the foundation upon which agriculture is built. Most contemporary crops are the products of evolutionary processes that began thousands of years ago, and that continue today as scientists harness emerging technologies to develop new crop varieties for a rapidly changing world. Current understanding of evolution under domestication is based primarily on annual plants, often self-compatible species that are propagated from seed each year. However, attention is refocusing on the development of perennial crops as concerns mount about a growing population, a changing climate, and sustainable agriculture. The domestication process in perennial fruit crops departs from that observed in annuals due primarily to differences in breeding systems (most perennial plants are outcrossing) and mode of reproduction (many perennial crops are clonally propagated). These differences have implications for two important aspects of perennial fruit crop domestication: 1) the extent and structure of population genetic variation in cultivated populations and their wild progenitors and 2) the genetic basis of agriculturally important traits. In order to better understand the hallmarks of perennial plant domestication, and to understand how perennial crop domestication might proceed in the future, we look to perennial fruit crops that have been evolving under domestication for thousands of years, including the apple (*Malus domestica*) and grape (*Vitis vinifera*) and their wild relatives. Using these examples, we explore the geographic and taxonomic mosaic of perennial crop domestication, the impact of genetic bottlenecks on variation in cultivated populations, crop-wild gene flow, and the genetic basis of phenotypic variation. We emphasize the importance of variation housed in wild-relatives for breeding fruits as well as rootstocks. These two iconic crops provide an important roadmap for exploring how best to conserve naturally occurring variation in perennial plant species and how to utilize it in plant breeding.

Keywords: perennial fruit crops, domestication, genetic variation, *Malus domestica*, *Vitis vinifera*, gene flow

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INTRODUCTION

The global significance of plant domestication cannot be overstated, as all modern food plants are the products of domestication, and future improved or new crops will necessarily undergo this process as humans strive to meet the needs of a growing population and a changing climate. Crop populations originate with the transfer of seeds or cuttings from natural settings to agricultural landscapes. As farmers identify individuals with traits that enhance crop production, and remove individuals with undesirable traits, this selective cultivation over the course of many generations causes crop populations to diverge morphologically and genetically from their wild progenitors. Although the domestication process may have started thousands of years ago for many plant species, it is not merely a phenomenon of the past. Today, ongoing domestication efforts occur primarily through targeted plant breeding programmes guided by modern genetic and genomic approaches. Contemporary domestication includes both the continued improvement of crops that originated thousands of years ago (like wheat and beans), as well as attempts to domesticate species that have not previously experienced artificial selection (like some species being used for biofuels). Indeed, the evolution of crop plants under domestication is the primary pathway to improving nutrition, yield, and resistance to abiotic and biotic stress under current and future predicted climates in the world's food plants.

The origins of domestication trace back nearly 10 000 years to the transition of humans from hunter-gatherer populations to agricultural societies. Several lines of evidence support multiple, independent origins of agriculture in at least seven geographically distinct regions (Vavilov, 1992). These agricultural centres are also "centres of domestication", geographic regions where the majority of crops originated, and that today retain important genetically variable and agriculturally valuable wild progenitors of modern crops. Originally, it was thought that for a given crop species, wild plants from a single geographic region at a single time point were taken into cultivation, followed by subsequent rounds of selection on cultivated individuals to generate the domesticated lineage (Zohary, 2012). However, more recent analyses suggest domestication likely involved multiple origins of a crop from wild populations over the course of many years, and perhaps from different geographic regions within a domestication centre (Brown *et al.* 2009). Today, domestication is viewed as a dynamic evolutionary process that occurs across broad spatial and temporal scales. Founder effects associated with the establishment of crop populations, ongoing artificial selection, and other evolutionary processes such as crop-wild gene flow, continue to contribute to the pace of plant evolution under domestication.

Agricultural societies are based primarily on domesticated annual plants that are usually self-fertile, and are propagated from seeds (Glémin and Bataillon, 2009). Not surprisingly, much of our current understanding of plant evolution under domestication is based the effects of genetic drift and artificial selection on these annual plant species (Hancock, 2005). For example, scientists have described a suite of traits in members of the grass family that change in predictable ways under domestication, including loss of shattering, synchronous flowering, larger fruits/grains, and more numerous fruits/grain per inflorescence (Glémin and Bataillon, 2009). Surveys have also shown that annual plant domestication is often accompanied by a domestication bottleneck (i.e. a reduction in genetic variation in cultivated populations relative to their wild relatives) (Miller and Gross, 2011). Finally, a large body of work has identified the genetic basis of many domestication traits, and this work shows that some traits are the result of single or few loci of large effect, while other domestication traits result from myriad, interacting loci of small effect (Olsen and Wendel, 2013). The majority of the calories consumed by humans are derived from annual grains and legumes; without doubt, these plants will continue to form the foundation of agriculture. However, as concerns mount about a changing climate and the sustainability of modern agriculture, attention is focusing on the potential of perennial plants, which offer promising options for food production while decreasing environmental impacts, and will likely play an increasingly important role in food production in the future.

PERENNIAL PLANTS HAVE BEEN IMPORTANT COMPONENTS OF AGRICULTURAL SOCIETIES SINCE THEIR INCEPTION

Perennial species make up between 35 and 80 percent of the total number of plant species domesticated in each of the major centres of origin (Meyer *et al.* 2012; Zohary, 2012). Perennial crops include a variety of herbaceous and woody plants that live for more than two years. These crops represent a broad range of plant families, and generally fall into two categories: 1) perennial species that are grown for their roots or other below-ground vegetative components, and 2) perennial species that are grown for their fruits. Although perennial plants that produce edible roots, tubers, or fleshy fruits have been cultivated for thousands of years (e.g. apple, grape, horseradish, potato), to our knowledge perennial grains have been conspicuously absent from agriculture (Van Tassel *et al.* 2010). The wide diversity of geographic and phylogenetic origins of traditional perennial crops means that each domesticated perennial is likely to have some unique features. However, it is possible to identify a general domestication syndrome associated with the evolution of perennial plants in response to artificial selection, which will be informative as breeders look towards domesticating other perennial species, including grains.

Current understanding suggests that annual plants preceded perennials in domestication, with perennial plant domestication reaching its first peak of activity 4 000 years ago (Meyer *et al.* 2012; Miller and Gross, 2011). Evidence suggests that this first peak of perennial domestication coincides with the widespread adoption of vegetative propagation. Just as naturally self-compatible annual plants appeared to be predisposed to domestication, similarly, perennial species that could be easily vegetatively propagated were among the first perennials to enter the domestication process. Interestingly, it seems that perennial grasses and legumes may have been overlooked by early farmers because natural selection had not favoured high seed production and ability to self-pollinate in perennial species to the extent that it had in annuals. In other words, annual grains may have been favoured over perennial grains historically because of their wild ancestors' higher seed productivity and/or greater ease of propagation (Van Tassel *et al.* 2010).

How do perennial plant species evolve under domestication? This question lies at the core of contemporary research programmes aimed at developing perennial grains and legumes; however, compared with annual crops, relatively little is known about how perennial species change in response to human selection. For example, what are the main traits that are under selection during perennial crop domestication? What is the extent and impact of domestication bottlenecks and artificial selection on genetic variation in cultivated populations of perennial plants? What is the potential for crop-wild or wild-crop gene flow in long-lived species? What is the genomic basis of domestication traits in perennials? Understanding evolutionary processes associated with perennial crop domestication will inform conservation strategies aimed at preserving genetic variation in cultivated populations and their wild progenitors, and will facilitate breeding efforts that are based on targeted selection within existing domesticates as well as the development of new domesticates.

WHAT MAKES PERENNIAL PLANTS DIFFERENT FROM ANNUAL PLANTS?

Aside from living for more than one year, perennial plants have several attributes that differentiate them from annual plants and that play a significant role in their capacity for evolution (McKey *et al.* 2010; Zohary and Spiegel-Roy, 1975). Perennial plants often have longer juvenile phases and lengthy reproductive cycles; where it takes an annual one year to grow from a seed and produce another seed, it could take a perennial plant many years before a germinated seed is capable of producing viable offspring. This duration means that it can take several years or even decades for a single generation to be completed. In a one-hundred year time period, an annual plant will have 100 cycles of sexual reproduction on which selection can act; in contrast, a perennial plant could have half that, or much less. In practical terms, this means that evolution could take much longer in perennials than annuals because it takes many more years to achieve the same number of sexual cycles. All things being equal, under this scenario we might expect domesticated perennials to show less divergence from their wild progenitors over time relative to annual plants, because fewer cycles for selection have occurred. However, many perennial plants exhibit stark morphological contrasts compared with their wild progenitors, suggesting that evolution, although operating over fewer sexual cycles, results in clear morphological changes over relatively few generations. This suggests that there may be unique aspects to perennial plants that are not regularly observed in annuals, and that are contributing their evolutionary potential and trajectories.

There are two other features of perennial crops that stand in stark contrast to annual plants, and both are related to reproduction. The first distinguishing feature of perennial species is that they tend to be obligate outcrossers and exhibit a range of mechanisms that prevent self-pollination and/or self-fertilization, including dichogamy, dioecy, or self-incompatibility, among others (Zohary and Spiegel-Roy, 1975). This is in contrast to the self-compatible systems found in most annual crops, either the result of evolution of self-compatibility under domestication or because annual crops were domesticated primarily from wild populations with the capacity for self-fertilization (note that some annual crops are predominantly outcrossing; however, many of the outcrossing annuals, such as maize and pearl millet are self-compatible). Generally speaking, outcrossing functions to increase heterozygosity within individuals, increase variation within populations, and decrease differentiation among populations as individuals exchange genes with plants from nearby populations or wild relatives. Interspecific gene flow likely plays an important role in the origin and evolution of perennial crops (Hughes *et al.* 2007 and see below). In crop populations where reproduction is based solely on sexual reproduction by seed, obligate outcrossing may slow the breeding process because all individuals, including those with the most desirable combination of traits, must hybridize with other individuals to yield seed. This can lead to the dilution of favoured traits in the cultivated population and increase the rate of masking of recessive alleles. On the flipside, obligate outcrossing in crops produces a nearly limitless amount of variation on which natural and artificial selection can act.

The second distinguishing feature of perennial plant reproduction relative to annuals has to do with how the plants reproduce in nature relative to how they are propagated in cultivation. Some perennial plants in natural populations reproduce clonally. This is thought to be a mechanism to enhance the likelihood of long-term viability of a genotype by producing multiple ramets, increasing the probability that at least some part of a genotype could transcend negative stochastic events that occur over the course of an extended lifespan (Vallejo-Marín *et al.* 2010). In cultivation, the majority of perennial crops are vegetatively propagated through layering, cuttings, grafting, or some combination of these three. Vegetative propagation addresses breeding challenges associated with long juvenile phases by instantaneously replicating genotypes with favourable traits. Because perennial crops are outcrossing and individuals are usually highly heterozygous, clonal reproduction replicates those individuals, thus resulting in populations comprising largely heterozygous individuals. However, clonal reproduction can lead to a low level of population variation and a high degree of population structure as entire populations can consist of one or a handful of genotypes. Increasing clonality within populations may be associated with reduced sexual reproduction and/or reduced fertility due to trade-offs associated with increased allocation of resources to vegetative growth, inbreeding depression, or mate limitation (McKey *et al.* 2010). Indeed, mate limitation in clonally propagated perennial plants is consistent with observed shifts toward self-compatibility, or in dioecious species, to hermaphroditism or parthenocarpy (McKey *et al.* 2010; Zohary and Spiegel-Roy, 1975)

In short, evolutionary processes in perennial plants are unique due in part to the combination of long juvenile phases, obligate outcrossing, and clonal reproduction. Even though annual crops undergo yearly cycles of sexual reproduction, seed production in annuals results largely from self-compatible individuals that produce relatively homozygous offspring. In contrast, while perennial crops have to wait years or decades for each cycle of sexual reproduction, the heterozygous individuals produced via outcrossing may be immortalized through clonal propagation. These differences in reproduction have significant implications for the extent and structure of population genetic variation in cultivated populations and crop wild relatives, and also for the genetic basis of agriculturally important traits.

IMPLICATIONS FOR POPULATION STRUCTURE AND THE GENETIC RESPONSE TO SELECTION

Perennial wild species are the sources from which perennial crops are descended. Several properties of perennial plant populations, including an outcrossed breeding system, hybridization with sympatric congeners, and life history strategies, promote variation within natural populations and decrease differentiation among populations (Petit and Hampe, 2006). Evolutionary processes operating in nature establish the highly diverse genetic foundation on which the domestication process is based. Understanding natural genetic variation in crop wild relatives is important for characterizing resources for breeding. In addition, the same evolutionary processes that shape variation in natural populations (outcrossing, intra- and interspecific gene flow, clonal reproduction) have implications for the genetic basis of evolution under domestication.

The trend for wild perennial species, including many of the wild relatives of domesticated perennials, is low population structure and high genetic variation (Petit and Hampe, 2006). These patterns are products of the characteristic perennial life history traits and breeding systems discussed above. In terms of population structure, for example, tree species are predicted and observed to be resistant to founder effects during the colonization of new habitats. This is partially due to the long juvenile phase of most trees, during which time the population can only grow via the arrival of new migrants (Austerlitz *et al.* 2000), and partially due to the rapid restoration of genetic diversity via long-distance pollen dispersal (Hampe *et al.* 2013). Because the changes in allele frequency that occur during mild or severe genetic bottlenecks are an important source of population differentiation, and thus population structure, many tree species (especially wind-pollinated species) will not exhibit population structure. These processes continue in existing natural populations within a species range, and are combined with a pattern of loss of homozygous individuals in population cohorts (Jolivet *et al.* 2013) to contribute to the high levels of genetic diversity seen in many tree species, including crop progenitors. High levels of gene flow often extend to interspecific hybridization among closely related species. Gene flow among close relatives appears common in long-lived species, and has been well documented in systems such as the oaks and poplars (Petit *et al.* 2004; Stolting *et al.* 2013). Nonetheless, tree populations can be vulnerable to the effects of habitat fragmentation, and some studies show that trees in long-term fragmented habitats either show signs of inbreeding or increased genetic structure among younger cohorts (Vranckx *et al.* 2012). This may have important implications for the wild relatives of some crop species, especially those in areas with a long history of human habitation and high population densities.

Interestingly, the high levels of gene flow among populations of tree species do not prevent populations within those species from responding to geographically variable selective regimes. Studies in wild species consistently show that populations are locally adapted along biotic and abiotic gradients within a species range, some of which are quite extensive (González-Martínez *et al.* 2006). In cases where the genetic bases of these adaptive traits have been documented, the underlying loci appear to be numerous and of relatively small effect in terms of the percentage of variation explained (Eckert *et al.* 2010). The population structure, genetic variation, and currently documented genetic basis of adaptive traits in natural tree populations have important implications for the process and genetic basis of domestication in perennial plants. First, the lack of genetic structure means that genetic analysis of crop relatives might be less likely to result in false associations due to linked variation. Genetic structure has presented a major impediment to accurately identifying the genetic basis of selectively advantageous traits in many annual systems (wild and domesticated). Second, if wild perennial populations can adapt to different selective pressures via many changes of small effect, and in the face of gene flow, then it is possible perennial crops may respond to artificial selection in a similar manner. This would stand in contrast to the genetic basis of domestication documented in many (although not all) annual crops, in which domestication traits are controlled by loci or genes of major effect (Purugganan and Fuller, 2009). Progress and challenges in the effort to identify and characterize the genetic basis of adaptation in perennial crops are detailed below.

WHAT CAN BE LEARNED FROM PERENNIAL CROPS THAT HAVE BEEN UNDERGOING DOMESTICATION FOR SEVERAL CENTURIES?

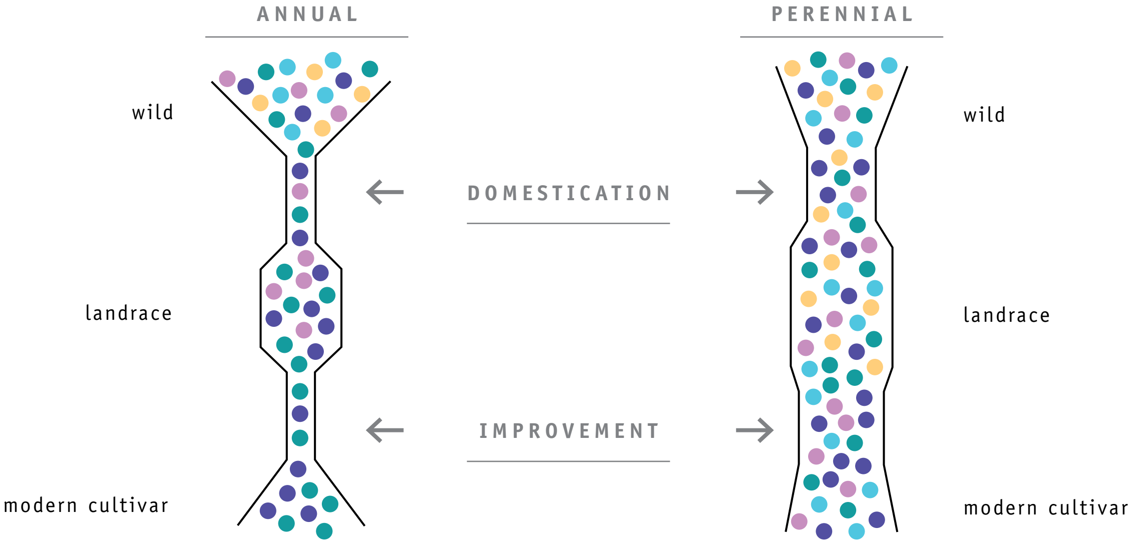
Research indicates that perennial crops originate and evolve in a fundamentally different way than annual crops, and these differences have important implications for crop breeding and improvement (McKey *et al.* 2010; Miller and Gross, 2011; Zohary and Spiegel-Roy, 1975). While more research on the topic is badly needed, current evidence indicates that multiple origins are the rule rather than the exception for perennials, with more than half of the perennial crops studied to date showing evidence of two or more origins (Miller and Gross, 2011). This is particularly interesting in light of the low levels of genetic structure detected in most of the wild relatives of perennial crops; the lack of structure should make estimates of multiple origins fairly conservative because there is not enough differentiation in most of the wild species' range to accurately detect distinct lineages. Moreover, the "more than half" estimate of multiple origins for perennial crops does not include the instances of multiple perennial crops of the same genus – at least six genera contain two or more domesticated species. Taken together, these trends may indicate that some species and genera of perennial crops should be considered good candidates for a new or re-domestication process, or simply for extensive improvement. It is certainly clear that desired traits in these polyphyletic crops can be assembled from a variety of starting points, so redeveloping these traits in a related species or from a new population within the same species should be feasible.

In addition, domesticated perennials appear to undergo crop-wild and wild-crop gene flow. Crop-wild gene flow is a broadly common phenomenon, and has been documented for annual and clonal crops (Ellstrand *et al.* 1999). In perennial crops, the best-documented cases are from some of the older domesticates (grape, olive, and apple), where gene flow has been demonstrated both from the crop into the wild species and vice versa. Gene flow from domesticated lineages into wild populations is an area of concern for conservation reasons; this scenario has been documented for apple and grape (Di Vecchi-Staraz *et al.* 2009; Gross *et al.* 2012). Hybridization between feral and domesticated olive may also be contributing to weed evolution in Australia (Besnard *et al.* 2007). However, there are also more positive outcomes in grapes and apples, were the wild species' contribution to the domesticated lineage may have been a key part of the domestication process, contributing to the development of unique varieties or facilitating the movement of a domesticated lineage into a new geographical region (Cornille *et al.* 2012; Lopes *et al.* 2009; Myles *et al.* 2011). Gene flow may well be more common than it appears to be based on existing studies, as it can be difficult to detect when there is insufficient genetic differentiation between the crop and its wild progenitor. Thus, it is likely that larger marker datasets (i.e. those resulting from next-generation sequencing approaches) will reveal more instances of gene flow in future studies. In light of the information in apple and grape, and in consideration of the propensity for multiple origins of several perennial crops, wild germplasm represents a powerful resource for perennial plant breeding. While the long juvenile phase of most perennial crops can justifiably make plant breeders cautious in the crosses they choose, the relative ease with which the domestication phenotype can be assembled (either from multiple starting points or in the face of gene flow from the wild species) suggests that the time investment may yield a high return.

Perennial crops appear to experience a relatively mild genetic bottleneck associated with domestication (Figure 1). While genetic bottlenecks vary in intensity from crop to crop, for annual species the average reduction in genetic variation during the domestication bottleneck is ~40 percent. This is in strong contrast to the average bottleneck in perennial crops, where the average reduction in diversity is only 5 percent (Miller and Gross, 2011). There are many factors that could contribute to the relatively mild genetic bottlenecks in perennial crops, including the aforementioned trends towards outcrossing (when sexual reproduction occurs), multiple origins of crop lineages, and crop-wild gene flow. Whatever the cause, the mild genetic bottlenecks mean that many domesticated perennials have high genetic variation, often close to what is seen in their wild relatives. The full extent of this variation has not yet been utilized in cultivation or breeding programmes (Myles, 2013; Myles *et al.* 2011). Ongoing efforts to preserve the range of variation under cultivation in living germplasm banks or repositories support an invaluable resource for crop improvement. It is also interesting to consider that the genetic bottleneck that accompanies annual plant domestication is one of the contributors to the phenotypic and genetic differentiation between the crop and the wild species. The loss of diversity during the bottleneck is random, and can lead to loss or fixation of alleles by chance alone; these changes in allele frequency allow the crop to be distinguished from the wild species based on genetic analysis. However, many domesticated perennials can also be effectively distinguished from their wild progenitors, *despite* a very mild bottleneck. Thus, the genetic and phenotypic differentiation must be attributed to other factors, including (both not limited to) artificial selection. Finally, from a plant breeding perspective, the mild genetic bottlenecks in perennial crops mean that genome scans designed to detect regions of low diversity (suggestive of genes under selection) will not be impeded by the confounding effects of a genetic bottleneck, as has been the case for some annual crops (e.g. Hamblin *et al.* 2006).

FIGURE 1. DOMESTICATION AND IMPROVEMENT BOTTLENECKS FOR ANNUAL VERSUS PERENNIAL CROPS

Different coloured circles represent variation in alleles or phenotypes present a given species undergoing domestication. The width of the funnel represents the relative population sizes during different stages of domestication. Variation is either lost during the domestication and improvement process in annual crops, or retained through these processes in perennial crops.



GENETIC BASIS OF DOMESTICATION TRAITS

Evolutionary biologists and plant breeders have pursued a variety of approaches to elucidate the genetic underpinnings of domestication traits in perennial crops. The primary tool used thus far has been QTL mapping, and it has been applied to a number of perennial crop systems (see Miller and Gross, 2011). When QTL maps are based on crosses between two cultivars, they are very useful for pinpointing the genetic basis of agronomically valuable traits that segregate within a domesticated lineage. However, they tell us little about the genetic basis of domestication traits – the traits that evolve during the domestication process and that differ between wild and domesticated plants. The general trend of the QTL mapping studies (either within the crop or in the few existing crop-wild crosses) indicates that domestication traits are underpinned by many QTL of small effect, although QTL of major effect have also been observed.

While QTL mapping has been very useful in the quest to identify loci associated with traits of agricultural importance with the goal of food improvement, it is also subject to several weaknesses. One of these is the high variability of QTL detection across populations and across years within populations (Crouzillat *et al.* 2000; Kenis *et al.* 2008). While this is expected to some extent (the expression of phenotypic traits in an orchard is dependent on environmental influences), the high variability makes it difficult to move forward to map-based cloning, a technique used to identify many domestication genes in annual plants. Efforts to clone the genes underlying these traits are also hindered by the fact that fine mapping requires analysis of hundreds of crossover events – the equivalent of a mapping population with at least 500 (and usually over 1 000) individuals. This is clearly not a realistic goal for every large-statured, perennial crop - the monetary and temporal investment required to maintain plants through their juvenile phase to maturity is beyond the reach of most individual researchers, and many institutions. Instead, investigations into domestication genetics and plant breeding are utilizing techniques that do not require mapping populations, but rely on existing variation and the power of massively parallel high-throughput sequencing techniques (i.e. "next generation sequencing" or NGS). Here we discuss the initial application of these techniques and their potential application to perennial crops in the future.

Genome-wide association studies (GWAS), also referred to as association mapping, is an excellent alternative to QTL mapping in perennial plants, because it takes advantage of the variation in existing populations (wild or domesticates), thus allowing breeders to skip the step of generating a new mapping population and simultaneously take full advantage of the large collections that exist for many perennial crops (Khan and Korban, 2012). However, there are two important considerations for the implementation of GWAS (Khan and Korban, 2012; Myles, 2013). One is that genetic structure or genetic differentiation within the surveyed population can lead to spurious associations. For example, the existing differentiation between wild and domesticated lineages means that a GWAS can be effectively carried out in *either* wild trees *or* domesticated trees – a GWAS conducted on a mixture of these two lineages would only reveal that all the wild phenotypes were in correlated with all the wild-specific markers. The second consideration is that linkage disequilibrium (LD) becomes a double-edged sword. Low levels of LD mean that significant correlations should be located in or (very close to) the gene controlling the trait of interest, but that it will require a very large number of markers to thoroughly cover the genome and actually capture these associations. High levels of LD can allow a large genome to be scanned using relatively few markers, but a significant association may still be very far from the functional gene or genomic feature. However, given the relatively low cost of generating SNP makers using NGS techniques, it seems unlikely that requiring a large number of markers will be a roadblock in the coming years. In light of this, it is fortunate that many perennial species have relatively low LD – this should make GWAS a powerful approach for elucidating the genetic underpinnings of phenotypic traits. In species with high LD, the targeted creation of QTL mapping populations that will generate recombinants in the genomic are of interest can complement the GWAS approach.

Association studies can also be carried out in a more targeted way if researchers have enough information to identify candidate genes that could contribute to the trait of interest. In this case, SNP markers can be genotyped in the region of interest, or the entire gene can be sequenced using targeted enrichment techniques (Cronn *et al.* 2012). This has been used to great effect in forest trees and fruit crops to confirm the association between candidate genes and traits of interest and to identify favourable alleles at these loci (Cevik *et al.* 2010; González-Martínez *et al.* 2007). This approach can be quite useful, but will ultimately be biased toward known genes. More 'agnostic' approaches like QTL mapping and GWAS still are necessary to identify genes that are not part of known families or have not been cloned in other species.

While both QTL mapping and GWAS are critical tools for dissecting the genetic basis of perennial crop domestication and improvement traits, they may not always be necessary to advance the crop domestication and improvement process from the perspective of breeders. Genomic selection (GS), the cousin of marker assisted selection (MAS), uses markers from across the genome to predict the phenotype of the plant in question (Heffner *et al.* 2009). This technique could be applied to the same types of populations used for GWAS studies, and utilizes the same type of markers, so the approaches are complimentary (Kumar *et al.* 2013; Myles, 2013). GS takes advantage of the fact that although many of the genes underlying traits are of small effect and difficult to map precisely, they still show detectable linkage with at least one marker. Once these patterns of linkage are established, it is possible to move forward to genotyping and phenotype prediction (based on a genotyped and phenotyped "training population"), allowing breeders to select seedlings for retention or removal long before they reach sexual maturity and set fruit for evaluation, thus speeding the process considerably.

All of these techniques have the potential to greatly advance the perennial crop improvement process, and can also increase our understanding of the genetic basis of perennial crop domestication. In particular, it is hoped that these techniques will allow crop breeders to take full advantage of the valuable genetic diversity present in most perennial domesticates. It should be noted, however, that while the high genetic diversity of most perennial crops will ultimately be an important resource to crop breeders, this same feature also presents a challenge for genomic approaches like GWAS and GS (discussed in Myles, 2013). Despite advances in sequencing technology and marker development, the option to start with inbred parents in crosses or in a population will greatly increase the power and accuracy of most NGS approaches. For example, SNP calls in heterozygous individuals are difficult because the heterozygous state has low support; more data is required to call these SNPs accurately. The overall diversity within perennials can also be difficult to accommodate, even if individuals are homozygous. This is because SNP data generated from NGS is too extensive to be checked manually, so its processing depends on the SNP and surrounding sequence matching a reference sequence almost exactly. This requirement is not always met in a species with high diversity, and results in many potentially variable sites being discarded. While analytical advances allowing for the imputation of missing data are being made, researchers and breeders working with these genetically diverse perennial crops must be prepared to generate an excess of data in order to have enough valid data to conduct their desired analyses.

WHAT CAN BE LEARNED FROM GRAPE AND APPLE?

In order to better understand the hallmarks of perennial plant domestication, and to predict how perennial crop domestication might proceed in the future, we look to two of the oldest and most economically important perennial fruit crops, apple (*Malus domestica*) and grape (*Vitis vinifera*), along with their wild relatives. This discussion follows on the heels of several recent papers that have drawn attention to these crops (Cornille *et al.* 2012; Meyer *et al.* 2012; Miller and Gross, 2011; Myles, 2013). Using these examples, we explore the geographic and taxonomic mosaic of perennial crop domestication, the impact of genetic bottlenecks on variation in cultivated populations, crop-wild gene flow, and the genetic basis of phenotypic variation. We emphasize the role of variation housed in wild-relatives for breeding fruits as well as rootstocks. These two iconic crops provide an important roadmap for exploring how best to conserve naturally occurring variation in perennial plant species and how to utilize it in plant breeding.

GRAPE

Cultivated grapevine (*Vitis vinifera* ssp. *vinifera*), the most economically important berry in the world, was domesticated from European grapevine (*Vitis vinifera* ssp. *sylvestris*) (This *et al.* 2006). The centre of diversity for *Vitis vinifera* appears to be in the Caucasus region, and multiple lines of evidence suggest that cultivated grapevines were domesticated from wild vines in this area (Grassi *et al.* 2006; Imazio *et al.* 2013; Myles *et al.* 2011; Pipia *et al.* 2012). Patterns of molecular genetic diversity point to a role for western European *V. vinifera* in the evolution of cultivated grapevine as well, either as a second source of cultivated materials (Arroyo-García *et al.* 2006), or as a participant in crop-wild gene flow in the area (DeAndres *et al.* 2012; Myles *et al.* 2011). The domestication process in grape is characterized by a shift from dioecious wild progenitors to hermaphroditic cultivars, the seedlessness resulting from parthenocarpy or stenospermocarpy (Cabezas *et al.* 2006), a broad domestication bottleneck with high levels of variation retained in cultivated populations, and rapid decay of LD (Myles *et al.* 2011). Extensive genetic variation in cultivated and wild grapevines have been confirmed in surveys of breeding collections (Aradhya *et al.* 2003; Myles *et al.* 2011), broad-scale analyses of natural grapevine diversity (Grassi *et al.* 2006) and regional analyses of wild populations in France, (Barnaud *et al.* 2009), Spain (DeAndres *et al.* 2012), and the Caucasus region (Pipia *et al.* 2012).

Although grapevine cultivation is based primarily on the European grapevine *V. vinifera*, other *Vitis* species play critical roles in grape production as well (Galet, 1979). Most *Vitis* species can be distinguished morphologically and genetically from one another (Aradhya *et al.* 2003; Miller *et al.* in revision; Péros *et al.* 2010; Zecca *et al.* 2012); however, the majority of subg. *Vitis* (the largest subgenus within *Vitis* and the one that includes the European grapevine) are interfertile. Interspecific hybridization has played an important role in the development of grapevine cultivars in some parts of North America (Ali *et al.* 2011): European grapevines do not grow well in the eastern and central United States due primarily to their susceptibility to native pests and pathogens. Early European colonists experimented with crossing *V. vinifera* and native North American *Vitis*, generating hardy hybrid grapevines that exist in vineyards in the eastern half of the United States today. In addition, North American grapevine species have contributed valuable rootstocks for the global grape industry (Galet, 1979; Peccoux, 2012). Widespread grafting of *V. vinifera* to North American species dates back to the mid-1800's when insects in the genus *Phylloxera* devastated the French grape industry (Sorensen et al. 2008). Starting with this crisis, North American grapevines have provided the foundation for rootstock development, and are the focus of research programmes working to elucidate molecular mechanisms and genetic underpinnings of abiotic and biotic stress resistance in rootstocks (Gong *et al.* 2011; Marguerit *et al.* 2012; Pavlousek, 2011; Polesani *et al.* 2012). Today, many vineyards consist of European *V. vinifera* grafted to North American *Vitis* species, including the river grape (*V. riparia*), the rock grape (*V. rupestris*), and Berlandieri's grape (*V. cinerea* ssp. *berlandieri*), and their hybrid derivatives (Galet, 1979).

Both grapevine scions and rootstocks are the focus of crop improvement efforts using molecular markers to facilitate selection, but both are the products of highly heterozygous, outcrossing populations that exhibit rapid LD decay. Given this, what is the genetic basis of phenotypic variation in grapevine? Traditional QTL analysis has been used to characterize genetic architecture of berry quality, yield, and pest/pathogen resistance. QTL studies identified a few loci of relatively large effect associated with variation in berry colour, berry weight, number of inflorescences per shoot, and seedlessness in table grapes (Cabezas *et al.* 2006; Costantini *et al.* 2008; Doligez *et al.* 2010), and phenological stages in wine grapes (Duchêne *et al.* 2012), also demonstrating that variation in a few regions of the genome is associated with traits of agricultural significance. In contrast, proanthocyanidin production has a more complex genetic basis with multiple loci of small effect contributing to phenotypic variation (Huang *et al.* 2012). An alternative to traditional QTL studies is GWAS, which makes use of existing germplasm collections, sidestepping the need to generate trait-specific mapping populations (Morrell *et al.* 2011; Myles *et al.* 2009). Given the extensive natural variation in *Vitis*, the outstanding germplasm collections that have been established for *Vitis* in Europe, North America, and elsewhere, and the developing genomic resources for this genus (Jaillon *et al.* 2007; Scalabrin *et al.* 2010), association mapping offers a promising approach for characterizing the genomic basis of phenotypic variation in grapes.

Another approach to exploring regions of the genome that are involved traits of agricultural importance involves transcriptomics, the analysis of the expressed portion of the genome. In grapevine, a growing body of work incorporates various methods of transcriptome analysis to identify genes that are active during key stages of fruit ripening, abiotic stress, or biotic stress. Pioneering work in this field analysed expressed sequence tags isolated from different plant organs, developmental stages, and cultivars to identify genes that were up- or down-regulated during various stages of grape growth (Goes Da Silva *et al.* 2005). Subsequent studies have characterized transcriptional activity during berry development (Ali *et al.* 2011), and have described genomic response to abiotic stress (Cramer *et al.* 2007; Liu *et al.* 2012; Tillett *et al.* 2011). Comparative transcriptomics studies across species have been particularly powerful in dissecting species or cultivar-level differences in pathogen susceptibility (Polesani *et al.* 2012) and flavonoid biosynthesis (Ali *et al.* 2011). Many of these studies used an Affymetrix gene chip that was developed for grapevine.

The transcriptome approach to identifying candidate genes associated with agriculturally important traits is only expanding with the advent of massively parallel sequencing of ribonucleic acid (RNA), (RNA-seq). RNA-seq offers an efficient, cost-effective way to access all species of transcript in a given tissue at a given time point, and can be used to describe both DNA sequence and transcript abundance (Wang *et al.* 2009). In grapevine, RNA-seq has been used to generate de novo transcriptomes for cultivars (Venturini *et al.* 2013), which can then be compared with reference genomes or transcriptomes of other cultivars facilitating the identification of cultivar-specific transcript. This promising approach is particularly powerful for clonally propagated perennial plants where individual genotypes live for multiple years and are replicated over diverse landscapes. For long-lived clones, RNA-seq offers the unparalleled opportunity to characterize temporal and spatial variation in a genotype's genomic response to whatever it may encounter.

APPLE

What is known about domesticated apple relative to the general features of perennial crop domestication? *Malus domestica* is one of the world's major fruit crops, is economically one of the most important, and is planted widely in the northern and southern hemispheres. As such, it has been the subject of intense study, and these studies show that apple demonstrates many of the major trends for perennial fruit crops detailed above. Genetic diversity in both wild and domesticated apple is very high, and individual plants are highly heterozygous (Cornille *et al.* 2012; Richards *et al.* 2009; Velasco *et al.* 2010). Wild populations of the progenitor, *Malus sieversii*, appear to have low population structure with high levels of outcrossing; this corresponds well with research on other wild tree species (Richards *et al.* 2009). Domesticated apple shows no sign of an improvement bottleneck, retaining the same high levels of diversity seen in its close wild relatives (Cornille *et al.* 2012). The results of one study indicate that this may be partially attributed to the influx of genetic variation from one of the close wild relatives, *Malus sylvestris* (Cornille *et al.* 2012). Gene flow from the domesticated species into *M. sieversii* has also been documented, which is an issue of possible conservation concern (Gross *et al.* 2012). As for many perennial crops, however, the broad genetic diversity of the crop and the wild species are not yet fully utilized or reflected in most modern breeding programme (other than introgression of disease-resistance genes) or in the active commercial orchards for this crop.

QTL mapping has been applied to several cultivar × cultivar crosses, and most domestication traits (mainly related to fruit quality) are controlled by loci of small effect (Kenis *et al.* 2008). Not surprisingly, these small-effect loci are inconsistent across populations and across years within a population, due to environmental effects and differences in genetic background. While these QTL studies have been enormously useful for MAS efforts dealing with disease resistance genes (which tend to have a larger effect) (King *et al.* 1999), a more targeted approach incorporating candidate genes has been necessary to identify and utilize alleles associated with fruit quality, and, more recently, growth habit (Baldi *et al.* 2012; Cevik *et al.* 2010). While these approaches are impressive, it is likely that important loci and genes remain undiscovered due to the lack of precision of QTL mapping and limited mapping population size used in most of these studies. GWAS approaches and genome scans for loci under selection offer promising avenues to identify these loci.

Both the breeding and genetics of apple were advanced with the resources building up to and culminating in the sequencing of the apple genome (‘Golden Delicious’ cultivar) followed by resequencing of 27 additional cultivars (Chagne *et al.* 2012; Velasco *et al.* 2010). While much work remains to curate the apple genome (a difficult task, due partially to the high heterozygosity), this has resulted in many advances. Analysis of SNPs developed based on these genomes indicate that LD in domesticated apples (outside of structured family populations) is low, which will make the link between significant markers in GWAS and the underlying gene more realistic than in a high LD species. Encouragingly, in the first GWAS approach for *Malus*, using a structured family population and 2 500 SNPs, several known candidate genes were recovered in the initial scans. Moreover, a GS approach to the same population indicate that fitting markers across the genome was effective in capturing phenotypic variation that is very difficult to track at the single-marker level (Kumar *et al.* 2013). This study also confirmed the difficulties inherent in working with a highly diverse species – the SNPs were based on an 8K SNP array, but only 2 500 were robust enough to be included in the final analysis. While even the number of SNPs used in this GS study may seem a daunting goal for non-model species, rapid advances in SNP generation technology and analysis are likely to level the playing field very rapidly. Instead, it is possible that the limiting resource for non-model crops might end up being the generation, maintenance, and phenotyping of large pedigreed populations such as nested association mapping (Kotoda *et al.* 2010) lines that are extremely useful for GWAS and GS studies. While these populations represent a substantial investment, they position researchers to immediately take advantage of developing technologies, and should be a priority for the research community.

REFERENCES

Ali, M.B., Howard, S., Chen, S., Wang, Y., Yu, O., Kovacs, L.G. & Qiu, W. 2011. Berry skin development in Norton grape: Distinct patterns of transcriptional regulation and flavonoid biosynthesis. *BMC Plant Biology*. 11: 7.

Aradhya, M., Dangl, G., Prins, B., Boursiquot, J., Walker, M., Meredith, C., Simon, C. 2003. Genetic structure and differentiation in cultivated grape, *Vitis vinifera* L. *Genetical Research*. 81: 179-192.

Arroyo-García, R., Ruiz-García, L., Bolling, L., Ocete, R., López, M., Arnold, C., Ergul, A., Söylemezo,˝lu.G., Uzun, H., Cabello, F., Ibáñez, J., Aradhya, M., Atanassov, A., Atanassov, I., Balint, S., Cenis, J., Costantini, L., Gorislavets, S., Grando, M., Klein, B., McGovern, P., Merdinoglu, D., Pejic, I., Pelsy, F., Primikirios, N., Risovannaya, V., Roubelakis-Angelakis, K., Snoussi, H., Sotiri, P., Tamhankar, S., This, P., Troshin, L., Malpica, J., Lefort, F. & Martinez-Zapater, J. 2006. Multiple origins of cultivated grapevine (*Vitis vinifera L. ssp. sativa*) based on chloroplast DNA polymorphisms. *Molecular Ecology*. 15: 3707-3714.

Austerlitz, F., Mariette, S., Machon, N., Gouyon, P-H. & Godelle, B. 2000. Effects of colonization processes on genetic diversity: Differences between annual plants and tree species. *Genetics*. 154: 1309-1321.

Baldi, P., Wolters, P.J., Komjanc, M., Viola, R., Velasco, R. & Salvi, S. 2012. Genetic and physical characterisation of the locus controlling columnar habit in apple (*Malus* × *domestica* Borkh.). *Molecular Breeding*. 31: 429-440.

Barnaud, A., Laucou, V., This, P., Lacombe, T. & Doligez, A. 2009. Linkage disequilibrium in wild French grapevine, *Vitis vinifera L*. subsp. *silvestris*. *Heredity*. 104: 431-437.

Besnard, G., Henry, P., Wille, L., Cooke, D. & Chapuis, E. 2007. On the origin of the invasive olives (*Olea europaea* L., Oleaceae). *Heredity*. 99: 608-619.

Brown, T.A., Jones, M.K., Powell, W., Allaby, R.G. 2009. The complex origins of domesticated crops in the Fertile Crescent. *Trends in Ecology & Evolution*. 24: 103-109.

Cabezas, J.A., Cervera, M.T., Ruiz-García, L., Carreño, J., Martínez-Zapater, J.M. 2006. A genetic analysis of seed and berry weight in grapevine. *Genome*. 49: 1572-1585.

Cevik, V., Ryder, C., Popovich, A., Manning, K., King, G. & Seymour, G. 2010. A *FRUITFULL*-like gene is associated with genetic variation for fruit flesh firmness in apple (*Malus domestica* Borkh.). *Tree Genetics & Genomes*. 6: 271-279.

Chagne, D., Crowhurst, R.N., Troggio, M., Davey, M.W., Gilmore, B., Lawley, C., Vanderzande, S., Hellens, R.P., Kumar, S., Cestaro, A., Velasco, R., Main, D., Rees, J.D., Iezzoni, A., Mockler, T., Wilhelm, L., Van de Weg, E., Gardiner, S.E., Bassil, N. & Peace, C. 2012. Genome-wide SNP detection, validation, and development of an 8K SNP array for apple. *PLoS One*. 7: e31745.

Cornille, A., Gladieux, P., Smulders, M.J.M., Roldán-Ruiz, I., Laurens, F., Le Cam, B., Nersesyan, A., Clavel, J., Olonova, M., Feugey, L., Gabrielyan, I., Zhang, X-G., Tenaillon, M.I. & Giraud, T. 2012. New insight into the history of domesticated apple: Secondary contribution of the European wild apple to the genome of cultivated varieties. *PLoS Genetics*. 8: e1002703.

Costantini, L., Battilana, J., Lamaj, F., Fanizza, G. & Grando, M. 2008. Berry and phenology-related traits in grapevine (*Vitis vinifera L.*): From Quantitative Trait Loci to underlying genes. *BMC Plant Biology*. 8: 38.

Cramer, G., Ergül, A., Grimplet, J., Tillett, R.L., Tattersall, E., Bohlman, M., Vincent, D., Sonderegger, J., Evans, J., Osborne, C., Quilici, D., Schlauch, K., Schooley, D. & Cushman, J. 2007. Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. *Functional & Integrative Genomics*. 7: 111-134.

Cronn, R., Knaus, B.J., Liston, A., Maughan, P.J., Parks, M., Syring, J.V., Udall, J. 2012. Targeted enrichment strategies for next-generation plant biology. *American Journal of Botany*. 99: 291-311.

Crouzillat, D., Ménard, B., Mora, A., Phillips, W. & Pétiard, V. 2000. Quantitative trait analysis in *Theobroma cacao* using molecular markers: Yield QTL detection and stability over 15 years. *Euphytica*. 114: 13-23.

DeAndres, M.T., Benito, A., Perez-Rivera, G., Ocete, R., Lopez, M., Gaforio, L., Munoz, G., Cabello, F., Zapater, J.M. & Arrroyo-Garcia, R. 2012. Genetic diversity of wild grapevine populations in Spain and their genetic relationships with cultivated grapevines. *Molecular Ecology*. 21: 800-816.

Di Vecchi-Staraz, M., Laucou, V., Bruno, G., Lacombe, T., Gerber, S., Bourse, T., Boselli, M. & This, P. 2009. Low level of pollen-mediated gene flow from cultivated to wild grapevine: Consequences for the evolution of the endangered subspecies *Vitis vinifera* L. subsp. *silvestris*. *Journal of Heredity*. 100: 66-75.

Doligez, A., Bertrand, Y., Dias, S., Grolier, M., Ballester, J., Bouquet, A. & This, P. 2010. QTLs for fertility in table grape (*Vitis vinifera L.*). *Tree Genetics & Genomes*. 6: 413-422.

Duchêne, E., Butterlin, G., Dumas, V. & Merdinoglu, D. 2012. Towards the adaptation of grapevine varieties to climate change: QTLs and candidate genes for developmental stages. *Theoretical and Applied Genetics*. 124: 623-635.

Eckert, A.J., van Heerwaarden, J., Wegrzyn, J.L., Nelson, C.D., Ross-Ibarra, J., Gonzalez-Martinez, S.C. & Neale, D.B. 2010. Patterns of population structure and environmental associations to aridity across the range of loblolly pine (*Pinus taeda* L., Pinaceae). *Genetics*. 185: 969-982.

Ellstrand, N.C., Prentice, H.C. & Hancock, J.F. 1999. Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics*. 30: 539-563.

Galet, P. 1979. *A Practical Ampelography*. Translated and adapted by Lucie T. Morton. Cornell University Press, Ithaca, NY.

Glémin, S., Bataillon, T. 2009. A comparative view of the evolution of grasses under domestication. *New Phytologist*. 183: 273-290.

Goes Da Silva, F., Iandolino, A., Al-Kayal, F., Bohlmann, M., Cushman, M., Lim, H., Ergul, A., Figueroa, R., Kabuloglu, E., Osborne, C., Rowe, J., Tattersall, E., Leslie, A., Xu, J., Baek, J., Cramer, G., Cushman, J. & Cook, D. 2005. Characterizing the the grape transcriptome. Analysis of expressed sequence tags from multiple *Vitis* species and development of a compendium of gene expression during berry development. *Plant Physiology*. 139: 574-597.

Gong, H., Blackmore, D., Clingeleffer, P., Sykes, S., Jha, D., Tester, M. & Walker, R. 2011. Contrast in chloride exclusion between two grapevine genotypes and its variation in their hybrid progeny. *Journal of Experimental Botany*. 62: 989-999.

González-Martínez, S.C., Krutovsky, K.V. & Neale, D.B. 2006. Forest-tree population genomics and adaptive evolution. *New Phytologist*. 170: 227-238.

González-Martínez, S.C., Wheeler, N.C., Ersoz, E., Nelson, C.D. & Neale, D.B. 2007. Association genetics in *Pinus taeda* L. I. Wood property traits. *Genetics*. 175: 399-409.

Grassi, F., Labra, M., Imazio, S., Rubio, R.O., Failla, O., Scienza, A. & Sala, F. 2006. Phylogeographical structure and conservation genetics of wild grapevine. *Conservation Genetics*. 7: 837-845.

Gross, B.L., Henk, A.D., Forsline, P.L., Richards, C.M. & Volk, G.M. 2012. Identification of interspecific hybrids among domesticated apple and its wild relatives. *Tree Genetics & Genomes*. 8: 1223-1235.

Hamblin, M.T., Casa, A.M., Sun, H., Murray, S.C., Paterson, A.H., Aquadro, C.F. & Kresovich, S. 2006. Challenges of detecting directional selection after a bottleneck: Lessons from *Sorghum bicolor*. *Genetics*. 173: 953-964.

Hampe, A., Pemonge, M.H. & Petit, R.J. 2013. Efficient mitigation of founder effects during the establishment of a leading-edge oak population. *Proceedings of the Royal Society B: Biological Sciences*. 280: 1764.

Hancock, J.F. 2005. Contributions of domesticated plant studies to our understanding of plant evolution. *Annals of Botany*. 96: 953-963.

Heffner, E.L., Sorrells, M.E. & Jannink, J-L. 2009. Genomic selection for crop improvement. *Crop Science*. 49: 1.

Huang, Y., Doligez, A., Fournier-Level, A., LeCunff, L., Bertrand, Y., Canaguier, A., Morel, C., Miralles, V., Veran, F., Souquet, J., Cheynier, V., Terrier, N. & This, P. 2012. Dissecting genetic architecture of grape proanthocyanidin composition through quantitative trait locus mapping. *BMC Plant Biology*. 12: 30.

Hughes, C.E., Govindarajulu, R., Robertson, A., Filer, D.L., Harris, S.A. & Bailey, C.D. 2007. Serendipitous backyard hybridization and the origin of crops. *Proceedings of the National Academy of Sciences.* 104: 14389-14394.

Imazio, S., Maghradze, D., Lorenzis, G., Bacilieri, R., Laucou, V., This, P., Scienza, A. & Failla, O. 2013. From the cradle of grapevine domestication: molecular overview and description of Georgian grapevine (*Vitis vinifera L.*) germplasm. *Tree Genetics & Genomes.* 9: 641-658.

Jaillon, O., Aury, J., Noel, B., Policriti, A, Clepet, C., Casagrande, A., Choisne, N., Aubourg, S., Vitulo, N., Jubin, C., Vezzi, A., Legeai, F., Hugueney, P., Dasilva, C., Horner, D., Mica, E., Jublot, D., Poulain, J., Bruyère, C., Billault, A., Segurens, B., Gouyvenoux, M., Ugarte, E., Cattonaro, F., Anthouard, V., Vico, V., Del Fabbro, C., Alaux, M., Di Gaspero, G., Dumas, V., Felice, N., Paillard, S., Juman, I., Moroldo, M., Scalabrin, S., Canaguier, A., Le Clainche, I., Malacrida, G., Durand, E., Pesole, G., Laucou, V., Chatelet, P., Merdinoglu, D., Delledonne, M., Pezzotti, M., Lecharny, A., Scarpelli, C., Artiguenave, F., Pè, M., Valle, G., Morgante, M., Caboche, M., Adam-Blondon, A., Weissenbach, J., Quétier, F. & Wincker, P. 2007. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature.* 449: 463-467.

Jolivet, C., Rogge, M. & Degen, B. 2013. Molecular and quantitative signatures of biparental inbreeding depression in the self-incompatible tree species Prunus avium. *Heredity*. 110: 439-448.

Kenis, K., Keulemans, J. & Davey, M. 2008. Identification and stability of QTLs for fruit quality traits in apple. *Tree Genetics & Genomes.* 4: 647-661.

Khan, M.A. & Korban, S.S. 2012. Association mapping in forest trees and fruit crops. *Journal of Experimental Botany*. 63: 4045-4060.

King, G.J., Tartarini, S., Brown, L., Gennari, F. & Sansavini, S. 1999. Introgression of the *Vf* source of scab resistance and distribution of linked marker alleles within the *Malus* gene pool. *Theoretical and Applied Genetics.* 99: 1039-1046.

Kotoda, N., Hayashi, H., Suzuki, M., Igarashi, M., Hatsuyama, Y., Kidou, S-I., Igasaki, T., Nishiguchi, M., Yano, K., Shimizu, T., Takahashi, S., Iwanami, H., Moriya, S. & Abe, K. 2010. Molecular characterization of *FLOWERING LOCUS T*-like genes of apple (*Malus* x *domestica* Borkh.). *Plant and Cell Physiology*. 51: 561-575.

Kumar, S., Garrick, D., Bink, M., Whitworth, C., Chagne, D., Volz, R. 2013. Novel genomic approaches unravel genetic architecture of complex traits in apple. *BMC Genomics.* 14: 393.

Liu, G., Wang, J., Cramer, G., Dai, Z., Duan, W., Xu, H., Wu, B., Fan, P., Wang, L. & Li, S. 2012. Transcriptomic analysis of grape (*Vitis vinifera L.)* leaves during and after recovery from heat stress. *BMC Plant Biology*. 12: 174.

Lopes, M.S., Mendonça, D., Rodrigues dos Santos, M., Eiras-Dias, J.E. & da Câmara Machado, A. 2009. New insights on the genetic basis of Portuguese grapevine and on grapevine domestication. *Genome.* 52: 790-800.

Marguerit, E., Brendel, O., Lebon, E., Van Leeuwen, C. & Ollat, N. 2012. Rootstock control of scion transpiration and its acclimation to water deficit are controlled by different genes. *New Phytologist.* 194: 416-429.

McKey, D., Elias, M., Pujol, B. & Duputié, A. 2010. The evolutionary ecology of clonally propagated domesticated plants. *New Phytologist.* 186: 318-332.

Meyer, R.S., DuVal, A.E. & Jensen, H.R. 2012. Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops. *New Phytologist.* 196: 29-48.

Miller, A.J. & Gross, B.L. 2011. From forest to field: Perennial fruit crop domestication. *American Journal of Botany*. 98: 1389-1414.

Miller, A.J., Matasci, N., Aradhya, M.K., Prins, B., Zhong, G.Y., Soimon, C., Buckler, E.S. & Myles, S. 2014. *Vitis* phylogenomics: hybridization intensities from a SNP array outperform genotype calls. *PLoS ONE* (in revision).

Morrell, P.L., Buckler, E. & Ross-Ibarra, J. 2011. Crop genomics: advances and applications. *Nature Publishing Group*. 13: 85-96.

Myles, S. 2013. Improving fruit and wine: what does genomics have to offer? *Trends in Genetics*. 29: 190-196.

Myles, S., Boyko, A.R., Owens, C.L., Brown, P.J., Grassi, F., Aradhya, M.K., Prins, B., Reynolds, A., Chia, J-M., Ware, D., Bustamante, C.D. & Buckler, E.S. 2011. Genetic structure and domestication history of the grape. *Proceedings of the National Academy of Sciences*. 108: 3530-3535.

Myles, S., Peiffer, J., Brown, P.J., Ersoz, E.S., Zhang, Z., Costich, D.E. & Buckler, E.S. 2009. Association mapping: Critical considerations shift from genotyping to experimental design. *Plant Cell*. 21: 2194-2202.

Olsen, K.M. & Wendel, J.F. 2013. Crop plants as models for understanding plant adaptation and diversification. *Frontiers in Plant Science*. 4: 290.

Pavlousek, P. 2011. Evalutaion of drought tolerance of new grapevine rootstock hybrids. *Journal of Environmental Biology*. 32: 543-549.

Peccoux, A. 2012. *Molecular and physiological characterization of grapevine rootstock adaptation to drought*. These pour le Doctorat de l'Université Bourdeuax 2. 186 pp.

Péros, J., Berger, G., Portemont, A., Boursiquot, J. & Lacombe, T. 2010. Genetic variation and biogeography of the disjunct *Vitis* subg. *Vitis* (Vitaceae). *Journal of Biogeography*. 38: 471-486.

Petit, R.J., Bodénès, C., Ducousso, A., Roussel, G. & Kremer, A. 2004. Hybridization as a mechanism of invasion in oaks. *New Phytologist*. 161: 151-164.

Petit, R.J. & Hampe, A. 2006. Some evolutionary consequences of being a tree. *Annual Review of Ecology, Evolution, and Systematics*. 37: 187-214.

Pipia, I., Gogniashvili, M., Tabidze, V., Beridze, T., Mgamkrelidze Gotsidridze, V., Melyan, G., Musayev, M., Salimov, V., Beck, J. & Schaal, B. 2012. Plastid DNA sequence diversity in wild grapevine samples (*Vitis vinifera* subsp. *sylvestris*) from the Caucasus region. *Vitis*. 51: 119-124.

Polesani, M., Bortesi, L., Ferrarini, A., Zamboni, A., Fasoli, M., Zadra, C., Lovato, A., Pezzotti, M., Delledonne, M. & Poverari, A. 2012. General and species-specific transcriptional responses to downy mildw infection in a susceptible (*Vitis vinfera*) and a resistant (*V. riparia*) grapevine species. *BMC genomics*. 11: 117.

Purugganan, M.D. & Fuller, D.Q. 2009. The nature of selection during plant domestication. *Nature*. 457: 843-848.

Richards, C.M., Volk, G.M., Reilley, A.A., Henk, A.D., Lockwood, D., Reeves, P.A. & Forsline, P.L. 2009. Genetic diversity and population structure in *Malus sieversii*, a wild progenitor species of domesticated apple. *Tree Genetics & Genomes*. 5: 339-347.

Scalabrin, S., Troggio, M., Moroldo, M., Pindo, M., Felice, N., Coppola, G., Prete, G., Malacarne, G., Marconi, R., Faes, G., Jurman, I., Grando, S., Jesse, T., Segala, C., Valle, G., Policriti, A., Fontana, P., Morgante, M. & Velasco, R. 2010. Physical mapping in highly heterozygous genomes: a physical contig map of the Pinot Noir grapevine cultivar. *BMC Genomics*. 11: 204.

Sorensen, W.C., Smith, E.H., Smith, J. & Carton, Y. 2008. Charles V. Riley, France, and Phylloxera. *American Entomologist*. 54: 134-149.

Stolting, K.N., Nipper, R., Lindtke, D., Caseys, C., Waeber, S., Castiglione, S. & Lexer, C. 2013. Genomic scan for single nucleotide polymorphisms reveals patterns of divergence and gene flow between ecologically divergent species. *Molecular Ecology*. 22: 842-855.

This, P., Lacombe, T. & Thomas, M. 2006. Historical origins and genetic diversity of wine grapes. *Trends in Genetics*. 22: 511-519.

Tillett, R., Ergul, A., Albion, R., Schlauch, K., Cramer, G. & Cushman, J. 2011. Identification of tissue-specific, abiotic stress-responsive gene expression patterns in wine grape (*Vitis vinifera L.*) based on curation and mining of large-scale EST data sets. *BMC Plant Biology*. 11: 86.

Vallejo-Marín, M., Dorken, M.E. & Barrett, S.C.H. 2010. The ecological and evolutionary consequences of clonality for plant mating. *Annual Review of Ecology, Evolution, and Systematics*. 41: 193-213.

Van Tassel, D.L., DeHaan, L.R. & Cox, T.S. 2010. Missing domesticated plant forms: can artificial selection fill the gap? *Evolutionary Applications*. 3: 434-452.

Vavilov, N.I. 1992. *Origin and Geography of Cultivated Plants* (English translation). Cambridge University Press, New York.

Velasco, R., Zharkikh, A., Affourtit, J., Dhingra, A., Cestaro, A., Kalyanaraman, A., Fontana, P., Bhatnagar, S.K., Troggio, M., Pruss, D., Salvi, S., Pindo, M., Baldi, P., Castelletti, S., Cavaiuolo, M., Coppola, G., Costa, F., Cova, V., Dal Ri, A., Goremykin, V., Komjanc, M., Longhi, S., Magnago, P., Malacarne, G., Malnoy, M., Micheletti, D., Moretto, M., Perazzolli, M., Si-Ammour, A., Vezzulli, S., Zini, E., Eldredge, G., Fitzgerald, L.M., Gutin, N., Lanchbury, J., Macalma, T., Mitchell, J.T., Reid, J., Wardell, B., Kodira, C., Chen, Z., Desany, B., Niazi, F., Palmer, M., Koepke, T., Jiwan, D., Schaeffer, S., Krishnan, V., Wu, C., Chu, V.T., King, S.T., Vick, J., Tao, Q., Mraz, A., Stormo, A., Stormo, K., Bogden, R., Ederle, D., Stella, A., Vecchietti, A., Kater, M.M., Masiero, S., Lasserre, P., Lespinasse, Y., Allan, A.C., Bus, V., Chagne, D., Crowhurst, R.N., Gleave, A.P., Lavezzo, E., Fawcett, J.A., Proost, S., Rouze, P., Sterck, L., Toppo, S., Lazzari, B., Hellens, R.P., Durel, C-E., Gutin, A., Bumgarner, R.E., Gardiner, S.E., Skolnick, M., Egholm, M., Van de Peer, Y., Salamini, F. & Viola, R. 2010. The genome of the domesticated apple (*Malus* x *domestica* Borkh.). *Nature Genetics*. 42: 833-839.

Venturini, L., Ferrarini, A., Zenoni, S., Tornielli, G.B., Fasoli, M., Santo, S.D., Minio, A., Buson, G., Tononi, P., Zago, E.D., Zamperin, G., Bellin, D., Pezzotti, M. & Delledonne, M. 2013. De novo transcriptome characterization of *Vitis vinifera* cv. Corvina unveils varietal diversity. *BMC Genomics*. 14: 41.

Vranckx, G., Jacquemyn, H., Muys, B. & Honnay, O. 2012. Meta-analysis of susceptibility of woody plants to loss of genetic diversity through habitat fragmentation. *Conservation Biology*. 26: 228-237.

Wang, Z., Gerstein, M. & Snyder, M. 2009. RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews: Genetics*. 10: 57-63.

Zecca, G., Abbott, J.R., Sun, W., Spada, A., Sala, F. & Grassi, F. 2012. The timing and the mode of evolution of wild grapes (*Vitis*). *Molecular Phylogenetics and Evolution*. 62: 736-747.

Zohary, D. & Spiegel-Roy, P. 1975. Beginnings of fruit growing in the Old World. *Science*. 187: 319-327.

Zohary, D., Maria, H. & Weiss, E. 2012. *Domestication of plants in the Old World*. Oxford University Press, New York, New York, USA.

15

DEVELOPMENT AND MARKETING OF PERENNIAL GRAINS WITH BENEFITS FOR HUMAN HEALTH AND NUTRITION

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ABSTRACT

"The Breeder's Dilemma - The Conflict Between Yield and Nutrition" addresses the challenge of breeding for highly nutritious grains when yield is the predominant selection criterion (Morris and Sands, 2006). Perennial grasses, in particular those that have already been used as food sources by indigenous peoples, offer an opportunity to develop sustainable and nutritious grain crops from genetic resources that have not been subjected to rigorous selection for yield. To date, our team has developed and commercialized two perennial grass crops and evaluated their nutrition profiles. Indian Rice Grass (IRG, *Achnatherum hymenoides*) was used by indigenous people in the western United States. Grain from this perennial grass was consumed as a staple as early as 7 000 years ago, long before maize was cultivated. The grains are smaller, and much higher in protein and essential amino acid content compared to wheat. These seeds shatter

and have a vernalization trait that suggests that they have not been domesticated in the modern agronomic sense. The grain can be ground into dark and flavourful, gluten-free flour that was marketed as Montina™. Another perennial grass product that has made it to market is Timtana™ flour, derived from Timothy grass seed (*Phleum pratense*). It is also high in protein, gluten-free and flavourful when used in baking. Both of these grains have a higher level of essential amino acids in their protein. With much of the world covered by perennial grains prior to agricultural development, there should be many more crops to develop as "new" emerging crops. A promising search strategy might be to focus on sites where baking ovens or ancient villages were once located. Collection of seeds of perennial plants from such locations may be particularly rewarding. Selection criteria might include several nutritional traits including high protein value, low glycaemic index, low phytic acid content, high omega-3 levels and absence of amylase-trypsin inhibitors.

Keywords: Indian rice grass, Timothy grass, nutrition, glycaemic index, phytic acid, omega-3, amylase-trypsin inhibitors

INTRODUCTION

A critical crop for the USA and world food production and nutrition is and will continue to be wheat. Valued for its superior bread making qualities, wheat is produced across the world and provides calories for a large number of people. It can be produced in dry climates with limited input. However, wheat is relatively low in protein nutritional quality, low in essential amino acids and has a high glycaemic index. In addition, wheat is closely associated with two emergent medical conditions: gluten intolerance (Sapone *et al.* 2011) and type 2 diabetes (Shulze *et al.* 2004; Gross *et al.* 2004). A long pressing problem in Montana is that there is no widely-used, profitable rotation crop for wheat (Chen *et al.* 2012). Legume production is increasing but the domestic and global market for pea and lentil is limited relative to the market for wheat. Rotation crops are important for optimum crop production because they break disease cycles and can greatly contribute to soil health and fertility. Development of alternate crops could increase rural and farm income, increase overall crop production, and have a significant impact on human nutrition and health. Our approach has been to search for high value crops that could serve as wheat alternatives, at a time when wheat prices have been high. To shift growers away from their traditional and subsidized crop and into planting an alternate crop, we needed to find a niche market where there was some value added advantage over wheat. This new market was the emergent gluten-free market (from US$200 million in 2007 to US$4.2 billion in 2013). Two of the four gluten-free crops that we have introduced into Montana agriculture are in fact perennial in habit.

Modern crop varieties have been often selected for high yield and transportation/storage stability. Increased yield equates to increased seed biomass. Increased biomass is more accurately described as increased carbohydrates (starch and fibre) and decreased protein (i.e. *The Breeders Dilemma*, Morris and Sands, 2006). Wheat, even soft white wheat, has also undergone extensive selection for increased gluten, valued for its superior bread- and pasta-making properties (Barro *et al.* 1997; Payne, 1987**)**. A growing number of consumers are unable to eat gluten. It is estimated that 6 percent of the USA population is gluten intolerant (celiac disease) or gluten sensitive (Fasano *et al.* 2011). Additionally, gluten is increasingly connected to diseases such as arthritis and neurological disorders (El-Chammas and Danner, 2011). The expanding gluten-free industry has responded by crafting food products from low-protein flour blends of rice, potato, cassava, and sorghum flours. There was insufficient attention paid to protein content or quality, even though gluten intolerant (celiac) customers actually require even more nutritional foods due to poor absorption of nutrients.

There are a large number of alternative crop candidates that should be considered for intensive breeding programmes; however those that are now available with improved nutrition, sustainable production and rotation potential are rather rare. Our strategy was to look at ancient grains consumed by indigenous peoples. So far, we have concentrated on an ancient grain crop that was consumed by indigenous Americans, Indian Rice Grass (IRG, Montina™, *Achnatherum hymenoides*). Seeds of this grass were found in prehistoric dwellings in Arizona (Bohrer, 1973). In addition, we have found that a pasture grass, Timothy (Timtana™, *Phleum pratense*) also produces a quality food grain. As with most perennial grains, yearly yields are lower than wheat, but once established, these grasses can yield for an extended number of years. Both are than detectible gluten content (Table 1). The essential amino acid content of Indian Rice Grass protein is much higher in comparison to spring wheat. (Table 2) As with most perennial grains, yearly yields are lower than wheat (Table 3) but once established, these grasses can yield for an extended number of years, reducing input costs including annual seeding, ground preparation, etc.

TABLE 1. NUTRITIONAL ANALYSIS (100 G SERVING)

	WHITE WHEAT FLOUR	MONTINA™ FLOUR (INDIAN RICE GRASS)	TIMTANA™ FLOUR (TIMOTHY GRASS)
Total Calories	364	380	300
Calories from Fat	8	27	50
Total Fat (g)	1	3	7
Saturated Fat (g)	0	0	0
Total carbohydrate (g)	76	70	63
Dietary fibre (g)	3	24	17
Protein (%)	10-12	17	17
Gluten	>5%	<0.5mg	<0.5mg

TABLE 2. PERCENT ESSENTIAL AMINO ACIDS IN PROTEIN: INDIAN RICE GRASS (IRG) VS. WHEAT

	IRG	WHEAT
Lysine	3.2	2.4
Methionine	2.1	0.5
Threonine	3.7	2.8
Isoleucine	2.8	5.3
Valine	3.5	2.1
Leucine	7.9	4.6
Arginine	9.3	2.2
Histidine	3.9	1.2
Phenylalanine	5.8	4.7
Total % Essential Amino Acids in Protein	**42.2**	**26.8**

TABLE 3. ANNUAL SEED YIELD

SEEDING RATE	YIELD	TYPE
Wheat (60# seeded/acre)	2 000-4 000 lbs/acre	Dryland or irrigated
Timothy (5# seeded/acre)	400-500 lbs /acre	Irrigated
IRG (4# seeded/acre)	100-200 lbs/acre	Dryland or irrigated

PERENNIAL CEREAL GRAINS

The Palaeolithic to Neolithic shift about 12 000 years ago was a shift toward production agriculture from a more nomadic hunting and gathering lifestyle (Wade, 2006; Wells, 2010). Concomitant with this shift was an increase in population sizes and inhabitation of areas that could support agrarian populations based on domestication of plants and animals. If the adaptable Palaeolithic lifestyle was sustainable in one sense, the Neolithic lifestyle was sustainable in a very different way. With agriculture, larger, denser populations could be sustained; culture could be more robust with far more complex social interactions (Wade, 2006; Wells, 2010).

It is important to recognize the importance of the role that annual cereal grains played in the intensification of agriculture. Such grains could be stored in granaries to tide over long periods of drought, pestilence, floods and overt predation depending on how well they were protected. The increased yield of annual plants may have facilitated establishment of sizeable reserves of grains, enabling a rapid selection of annual plants that were palatable, predictable in harvest date (determinant floral type), non-shattering, and yield-responsive to water added through irrigation or by late rainfall (Wells, 2010). It is not known why perennial grains were excluded from this series of developments. One might surmise that a population of annual grains might have a higher rate of change under strong annual selection than would a population of perennial grains. Also, any selection for yield after only one season would tend to favour an annual growth type as perennial type plants would be conserving energy in their root and crown systems for the next season. This subject is extensively reviewed (Van Tassel *et al.* 2010).

The rapid change in selection was probably influenced heavily by certain “seed villages” where a culture developed around selection of a mixed population (landraces) of diverse plant phenotypes to reflect the variance in growing conditions, disease and pest predation from year to year. These seed villages, probably the source of landraces of crops, gave rise to selection of favourable plants in terms of agronomic characteristics including disease and pest resistance (Harlan, 1957; Berg, 1992). They have served as important sources of germplasm for modern pure line monoculture breeding efforts in many centres of origin. For example, in the horn of Africa, North Africa, and throughout the Near East, such landraces are still grown and are favoured probably due to their reliable mixture of genotypes locally adapted to pests and diseases, although the yields are often not as high as those of improved cultivars (Ceccarelli *et al.* 1987).

The genetic flexibility of landraces has been largely replaced with the genetic flexibility of plant breeding. Plant breeding programmes are highly effective in combining favourable traits and modern breeding programmes have led to the Green Revolution, touted as saving millions of lives from certain starvation throughout the world. While yield has increased dramatically, the mineral nutrition in wheat has gone down in the past 160 years (Fan *et al.* 2008) There were bound to be some trade-offs from this intensified yield-driven, large-scale monoculture of just a few staple annual crops, including loss of plant diversity and reduction in protein. Perhaps these trade-offs can now be mitigated with a greater mindfulness of sustainability through water utilization and nutrient recycling, integrated pest management, and greater attention to human nutrition (Sands *et al.* 2009). One approach, the turn to perennial crops, may reduce inputs including the cost of seeds and fallow ground erosion. Several factors need to be considered in selection of perennial crops with a priority on human nutrition. The longer a plant is in the soil, the more exposure it has to predation by insects and rodents. This can be a problem, needing a solution through biocontrol or management practices. However, it can also suggest why perennial grains could be a good source of resistance traits for annual plant breeding development. In terms of nutritional value, it takes considerably more metabolic energy for a plant to produce a gram of protein than to produce a gram of starch. These are some of the interconnected factors that probably lead to an inverse relationship between yield and nutritional value (Morris and Sands, 2006; Sands *et al.* 2009). Perhaps selection for agronomic traits has had minimal impact on most cultivated perennial grains and no impact in many ancient grains, leaving their nutritional attributes intact.

HIGH NUTRITIONAL VALUE IN CEREAL GRAINS: A GOAL CONSTRAINED BY PLANT BIOLOGY?

Seeds are perceived as rich and compact sources of nutrition. However, for seeds to meet the needs of their own survival and plant reproduction, they have trade-offs that result in traits that are incompatible with or antagonistic to human nutritional needs. Plant seeds evolved to survive and cycle to the next generation, carrying adequate supplies of energy and major minerals. They polymerize all small molecules. This strategy is based on the phenomenon that the colligative (osmotic) effect of a small molecule is the same as that of a large polymer. If the seed contained too many "free" small molecules, the embryo could not survive their osmotic effect. Oils, insoluble compounds such as phytic acids that tie up zinc and iron, and hemicelluloses, starches and proteins solve this problem for seeds, thereby providing energy, trace elements and nitrogen to the embryo upon germination. Plants need only an initial nitrogen source from storage proteins, as they have a complete retinue of amino acid biosynthetic enzymes to re-synthesize all 20 amino acids. In contrast, animals can only synthesize ten, hence non-essential amino acids (Block and Bolling, 1945). The essential amino acid biosynthetic pathways are totally absent from animals. The essential amino acids are synthesized in plants and microbes and must be consumed by animals. The essential amino acid families are the aspartate family (lysine, methionine, and threonine), the branched chain amino acids (leucine, isoleucine, and valine) and the aromatic amino acids (phenylalanine, tyrosine, and tryptophan). Arginine is also essential (Block and Bolling, 1945).

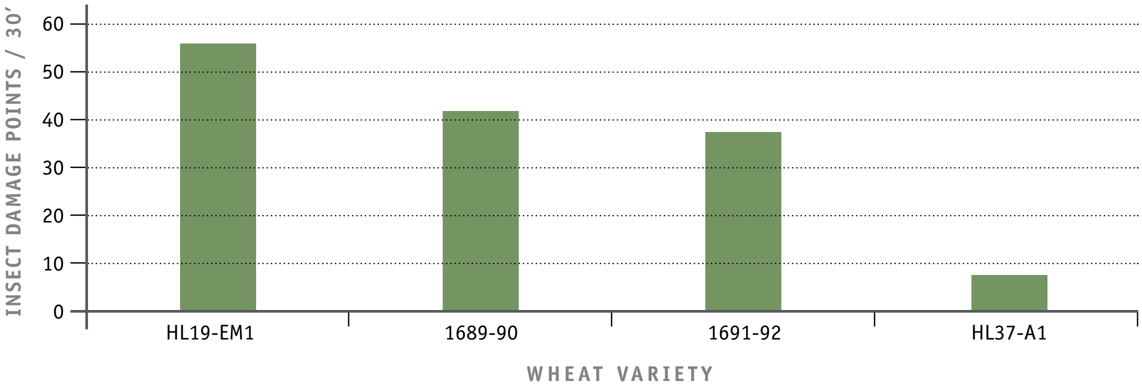
Throughout history, cereal grains have been regardwed as energy sources, (calories), and plant selection has proceeded accordingly. This view has resulted in selection of high yielding varieties (high starch i.e. calories) and lower protein. Furthermore the proteins in annual wheat, rice, barley, maize, sorghum and millet are imbalanced heavily in favour of non-essential amino acids (Ponter and Sauvant, 2004). Plants regulate the synthesis of these amino acids and have complex feedback systems to prevent overproduction. A case in point is lysine. From the standpoint of humans and animals; lysine is the most nutritionally limiting amino acid in cereal grains. To further complicate the nutritional picture, intensive breeding for pest and disease resistance may have resulted in selection of grains that are replete with families of small peptides that function as amylase trypsin inhibitors inhibiting digestive enzymes. These small peptides can drive intestinal inflammation and reduce nutrient absorption, especially in individuals afflicted with celiac disease (Junker *et al.* 2012). In our minds, the notable shortcoming of the aforementioned cereal grain intensification has been the lack of attention to human nutrition.

To remedy the nutrition crisis, we have identified several approaches outlined below that could be further developed: improving the nutritional quality of plants through intensified selection of specific amino acids, adding nutrients through fermentation of specific microbes, and identification of new perennial grasses that are high-protein, low-glycaemic and gluten-free.

IMPROVING THE NUTRITIONAL QUALITY OF FOOD STAPLES

To directly improve the nutritional quality of plants, we selected a series of high-lysine lines from a wheat population (18 years of selection) (Bright and Shewrey, 1983) and tested these lines for agronomic traits in plant breeders' field plots. A group of animals (aphids, grasshoppers, mice deer and antelope depending on location) devastated our cultivars in heavy preference over normal lines. Compared to their wild-type parent line (HL37-A1) they are favoured by insects and rodents, presumably because of their nutritional content (Figure 1) (Morris *et al.* 2006).

FIGURE 1. INSECT PREDATION ON 3 HIGH-LYSINE LINES COMPARED TO THE NORMAL LYSINE LINE HL37-A1



Source: Morris *et al.* 2006

This observation corroborates with our earlier work on chicken nutrition where we discovered that three-day old baby chicks discriminate against a zero lysine diet in favour of the same diet formulation with added lysine (Newman *et al.* 1984; Newman and Sands, 1983). The similar type of finding was reported (Osborne and Mendel, 1914), a century ago. They reported that rats did not grow on a wheat gliadin diet unless lysine was added. We know of a plant breeder who has simplified selection of nutritional traits simply by letting barn dwelling rodents select preferentially (i.e. eat) for nutritious lines. The basic concept is that a limiting factor (see Liebig's law of the limiting factor (Hardin, 1995) is still an operating paradigm in the animal feed industry. One important note, with respect to high-lysine wheat lines and probably high vitamin A rice lines, is that they are not yet commonly found in production agriculture (Morris *et al.* 2006). The increased predation on high-lysine lines will be very problematic unless the predation problem can be resolved. We speculate that such high nutrition lines might be used as trap crop loci to draw pests away from the desired crop. The best chance for high-lysine wheats and other similarly selected grains, if they are ever to reach the consumer, might be if they are crossed with high yielding advanced lines that have as a driver some particularly needed selection trait such as herbicide or rust resistance.

A decidedly different approach to plant based nutrition was tried by our group at Montana State University in the early 1980s. We constructed a DNA sequence designed to code for a highly nutritious protein that could be used to balance cereal grain diets (Jaynes *et al.* 1985). This synthetic protein was very high in lysine (22 percent), methionine (16 percent), and 10 percent each threonine, isoleucine and tryptophan. The DNA sequence was used to transform potato and the protein quality of the resulting transgenic potato was improved (Yang *et al.* 1989). We would hope that at some future point in time, the seed storage proteins of staple crops will be replaced with a new generation of designed, highly nutritious proteins as first demonstrated and described above by Jaynes *et al.* 1985.

Currently, we have selected and developed varieties of oat with higher levels (18-22 percent versus 12-13 percent) of protein. We have further selected these varieties for short stature to facilitate rapid visual identification and rogueing out of wheat and barley volunteer plants that contain gluten. This system has enabled production and commercialization of high-protein, gluten-free oatmeal and oat products.

APPROACHES TO IMPROVING NUTRITION FROM PLANT-BASED FOODS BY FERMENTATION WITH MICROBES SELECTED FOR EXCRETION OF SPECIFIC NUTRIENTS

As plant scientists, our strong interest in human nutrition has led us down several different avenues of research and development, including fermentation, forced selective breeding, review of undeveloped Palaeolithic grains, and high through-put selection of mutants. With regard to perennial grains, use of selected traditional fermenters can overcome the shortcomings of a particular grain. In our efforts to improve the nutritive value of both perennial and annual cereals, we have identified high phytic acid (binding zinc and iron), low quality protein and high glycaemic acid carbohydrates as high priority challenges. Our first approach to improving human nutrition did not actually involve plants directly. Fermentation has been a traditional means to preserve foods (wine, pickles, etc.) or to enhance flavour and texture (breads, yogurt, etc.). Foods can either be fermented with a known inoculum (e.g. yeast or sourdough starter) or with airborne inocula. In either case, the fermentation conditions are set up to favour the desired fermenting microbe.

Given that lysine is a limiting amino acid in many cereal based diets (Osborne and Mendel, 1914; Ponter and Sauvant, 2004), we selected two different high lysine-excreting bacteria (*Lactobacillus plantarum* and *Lactobacillus fermentum*). These bacteria are used for the fermentation of vegetables, dairy products and sourdough breads. We used an intensive selection procedure exposing these wild-type bacteria to higher and higher concentrations of toxic lysine analogues and selecting survivors (Sands and Hankin, 1974; Megeed and Sands, 2002). The survivors overcame the toxic analogues by overproducing lysine. When the lysine-overproducing *lactobacilli* were used to ferment dough, they continued to overproduce lysine, significantly increasing the lysine content of the resulting bread. The microbes could also be used to increase the lysine content of fermented vegetables or animal feed (e.g. silage). This strategy enabled fermented vegetables and cereal-based foods to be enriched in lysine regardless of the food or grain variety. The technology was also used to select lysine-excreting strains of yeast for bread production. It takes less time to select for such microorganisms than to improve lysine content of plants via breeding, with an estimated time of intensive repeated selection of 8 months for bacteria. A similar selection for either an enhanced annual or perennial plant would take years. These microbial strains and the methods are and have been available, but there has not yet been any widespread adoption. Commercial bacterial products used for food fermentation are generally touted for their organoleptic and probiotic properties, and not their excellent nutritional quality. Similarly, commercially available bread yeasts are promoted for their reliability and fast action, and price, not for the boost of lysine content or other important nutrients that they could deliver.

A HISTORICAL APPROACH TO HUMAN NUTRITION

As stated above, maybe we really need to step back and look at ancient grains and ancient peoples. Migrant populations depended upon what they could find. If meat was available, it was consumed. But if it was scarce, other sources of nutrition, primarily plants, were found. We tried to identify the ancient plants and to determine how to produce them. As mentioned earlier, the first plant that we worked with was IRG. The meal ground from seeds of this grass is high in protein, fibre, and flavour, with no trace of gluten. Grown as a perennial grass in the absence of gluten containing grains, the seed has been ground into flour and sold as Montina™, a gluten-free high-protein product for baking. The use of added gums (xanthan or guar) gives the bread the lift normally provided by gluten. The lesson learned here, with an admitted sample size of only one, is that ancient food grains, from before the plant breeding revolution, may be a worthwhile source of nutrition. We observe two types of evidence that this plant is not domesticated: the seeds require vernalization and seed shattering has not been eliminated. These two traits are not associated with domesticated grains (Wells, 2010). On the basis of this experience we strongly suggest that the search for unexploited grains is a productive strategy for identifying new annual and perennial grain crops.

Our second entry into the high protein gluten-free market niche was Timothy grass seed, trade marked as Timtana™. Timothy (*Phleum pratense*) was introduced into North America where it is established as a highly desired pasture grass. It is not known if grain from this grass was traditionally collected and consumed by people. There is a strong market for this small seeded perennial plant in the equine industry. To our surprise, no one had attempted to grind the seed into flour for human consumption. Timothy seed produced in isolation from gluten-containing cereals delivers excellent stand-alone or mixing flour for all manner of bread products, again is high in protein, flavour and fibre and gluten-free (Table 1, 2, and 3). It is the latter trait that has established this product in a high value niche market. Both Timothy grass and Indian rice grass are perennial and once established they have reduced water and fertilizer needs as compared with their annual counterparts.

Glycaemic Index

Protein malnutrition is a problem in much of the world (de Onis and Blossner, 2003). Additionally, an ever growing segment of the world is obese. Overall, of the world's adult population in 2005, 7.7 percent of men and 11.9 percent of women were obese and these percentages are projected to be increasing through 2030 (Kelly *et al.* 2008). Obesity is not in itself indicative of nutrition. It is indicative of over-consumption of calories especially in the form of starch. Most of our modern crops are selected for yield and the most efficient way for a plant to increase seed size is to increase storage starch relative to storage protein. In energetic terms, carbohydrates are less expensive to synthesize than protein. This is one reason why high protein wheat demands a premium price over lower protein wheat. In particular, plumper seed has a higher ratio of branched starch or amylopectin. Amylopectin, the branched form of starch, is rapidly digested, quickly releasing glucose (high glycaemic index), leading to that notable afternoon slump (Berti *et al.* 2004). This rapid spike in glucose is a real problem for diabetics. In contrast, amylose or straight starch is digested more slowly and the glucose spike is flattened. We suggest that we need to develop staple crops with lower glycaemic indexes (perhaps by reducing the GI to 50 percent of what they are now). This niche market could be even larger than the gluten-free market. Perennial grasses, with smaller seed sizes and less starch would be a good place to look for inherent low-glycaemic traits.

Overview

The requirements for the proper balance of essential amino acids needed for optimal nutrition have been known for nearly a century (Osborne and Mendel, 1914). It is time for a more proactive nutrition approach from plant science. There is evidence that valuable ancient food sources included perennial grains (Bohrer, 1973). Both Montina™ and Timtana™ are small seeded perennial grains. Perhaps the small seed size, relative to the major staple crops, is important in that the grain has to provide the plant with more nutrients per gram, and small seeds might offer a reduced target for predation. There are numerous molecular diagnostic products of basic research in plant genetics and biochemistry and tools available to implement improvement of crops relative to human nutrition. Given the advances in human biochemistry and physiology, we expect to see multidisciplinary linkages established to improve human nutrition relative to dietary components. Protein malnutrition should be a major target of plant geneticists. Plant breeders, by addressing these essential aspects of human nutrition, can fulfil the true needs of some populations that are not currently attaining their potential.

Perennialization as an approach to more sustainable agriculture might, in certain instances, turn the tide. However, there will be an uphill battle if yields are the principle "sine qua non" measure of success. Pests, weeds, and disease build-up in perennial systems will have to be addressed, perhaps with marker-assisted breeding, with multiline (mixed genotypes) approaches, with genetic engineering, and perhaps with pesticides either synthetic or biorational based measures. Perennial grains have their intrinsic sustainability values and advocates, in that they might reduce input costs. For example, in places where there are two rainy seasons, as in East Africa, the rattoon cutting of maize and/or sorghum after the long rains might lead to lower input costs and more erosion control and a real jump-start for the ensuing short rainy season, if weeds can be controlled. Perennial crops might be more sustainable in terms of soil holding, preventing bare ground wind and flood erosion, and lower input costs. They might need borrowed traits for disease and insect resistance from the existing intense annual plant breeding efforts. The strong suit of perennial crops might be that they could provide an input of enhanced human nutrition in addition to the environmental advantages that perennial crops can render.

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REFERENCES

Barro F., Rooke L., Bekes F., Gras P., Tatham A.S., Fido R., Lazzeri P.A., Shewry P.R., Barcelo P. 1997. Transformation of wheat with high molecular weight subunit genes results in improved functional properties. *Nature Biotechnology*. 15: 1295-1299.

Berg, T. 1992. Indigenous knowledge and plant breeding in Tigray, Ethiopia. *Forum for Development Studies*. 1:13–22.

Berti, C., Riso, P., Monti, L.D. & Porrini, M. 2004. In vitro starch digestibility and in vivo glucose response of gluten-free foods and their gluten counterparts. *European Journal of Nutrition*. 43, 198-204.

Block, R.J. & Bolling, D. 1945. The amino acid composition of proteins and foods. Analytical methods and results. *Yale Journal of Biology and Medicine*. 17: 580.

Bohrer, V. 1973. The prehistoric and historic role of the cool-season grasses in the Southwest. *Economic Botany*. 29:199-207.

Bright, S.W.J. & Shewry, P.R. 1983. Improvement of protein quality in cereals. *CRC Critical Reviews in Plant Sciences*. 1: 49-93.

Ceccarelli, C., Grando, S. & Van Leur, J.A.G. 1987. Genetic diversity in barley landraces from Syria and Jordon. *Euphytica*. 36: 389-405.

Chen, C., Neill, K., Burgess, M. & Bekkerman, A. 2012. Agronomic benefit and economic potential of introducing fall-seeded pea and lentil into conventional wheat-based crop rotations. *Agronomy Journal*. 104: 215-224.

de Onis, M. & Blossner, M. 2003 The World Health Organization global database on child growth and malnutrition: methodology and applications. *International Journal of Epidemiology*. 32(4): 518-526.

Dunmire, W.W. & Tierney, G.D. 1997. *Wild plants and native peoples of the Four Corners*. Museum of New Mexico Press, Santa Fe.

El-Chammas, K. & Danner, E. 2011. Gluten-free diet in nonceliac disease. *Nutrition in Clinical Practice*. 26: 294-299.

Fan, M-S., Zhao, F-J., Fairweather-Tait, S.J., Poulton, P.R., Dunham, S.J. & McGrath, S.P. 2008. Food chain evidence of decreasing mineral density in wheat grain over the last 160 years. *Journal of Trace Elements in Medicine and Biology*. 22: 315–324.

Gross, L.S., Li, L., Ford, E.S. & Liu, S. 2004. Increased consumption of refined carbohydrates and the epidemic of type 2 diabetes in the United States: an ecologic assessment. *The American Journal of Clinical Nutrition*. 79: 774-779.

Hardin, G. 1995. *Living within Limits: Ecology, Economics, and Population Taboos*. Reprint Oxford University Press, USA. pp. 207.

Harlan, H. 1957. *One Man's Life With Barley*. Exposition Press. New York, USA.

Jaynes, J.M., Langridge, P., Anderson, K., Bond, C., Sands, D., Newman, C.W. & Newman, R. 1985. Construction and expression of synthetic DNA fragments coding for polypeptides with elevated levels of essential amino acids. *Applied Microbiology and Biotechnology*. *21*(3-4): 200-205.

Jenkins, D.J.A., Kendall, C.W.C., Augustin, L.S.A., Fransceschi, S., Hhamidi, M., Mmarchie, A., Jenkins, A.L. & Axelsen, M. 2002. Glycemic index: overview of implications in health and disease. *The American Journal of Clinical Nutrition*. 76: 266S-273S.

Junker, Y., Zeissig, S., Kim, S.J., Barisani, D., Wieser, H., Leffler, D.A., Zevallos, V., Libermann, T.A., Dillon, S., Freitag, T.L., Kelly, C.P. & Schuppan, D. 2012. Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4. *Journal of Experimental Medicine*. 209: 2395-2408.

Kelly, T., Yang, W., Chen, C.S., Reynolds, K. & He, J. 2008. Global burden of obesity in 2005 and projections to 2030. *International Journal of Obesity*. 32: 1431–1437.

Megeed, M.A.E. & Sands, D.C. 1989. *Methods and compositions for improving the nutritive value of foods via* Lactobacillus fermentum. U.S. Patent 4,889,810.

Morris, C. E. & Sands, D.C. 2006. The breeder's dilemma - yield or nutrition? *Nature Biotechnology*. 24: 1078-1080.

Newman, R. K. & Sands, D. C. 1983. Dietary selection for lysine by the chick. *Physiology & Behavior*. 31: 13-19.

Newman, R.K., Sands, D.C. & Scott, K. 1984. A microbiological approach to nutrition. *Journal of the American Dietetic Association*. 84: 820-821.

Ogle, D., St. John, L. & Jones, T. 2013. *Plant Guide for Indian Ricegrass (Achnatherum hymenoides)*. USDA-Natural Resources Conservation Service, Aberdeen, Idaho.

Osborne, T.B. & Mendel, L.B. 1914. Amino acids in nutrition and growth. *Journal of Biological Chemistry*.17: 325-349.

Payne, P.I. 1987. Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. *Annual Review of Plant Molecular Biology*. 38: 141-153.

Ponter, A. & Sauvant, D. 2004. *Tables of composition and nutritional value of feed materials: pigs, poultry, cattle, sheep, goats, rabbits, horses and fish*. Wageningen Academic Publishers.

Sands, D.C. & Hankin, L. 1976. Fortification of foods by fermentation with lysine-excreting mutants of lactobacilli. *Journal of Agricultural and Food Chemistry*. 24: 1104-1106.

Sands, D.C., Morris, C.E., Dratz, E.A. & Pilgeram, A.L. 2009. Elevating optimal human nutrition to a central goal of plant breeding and production of plant-based foods, Review. *Plant Science*. 177: 377–389.

Sapone, A., Lammers, K.M., Casolaro, V., Cammarota, M., Giuliano, M.T., De Rosa, M., Stefanile, R., Mazzarella, G., Tolone, C., Itria, Russo, M., Esposito, P., Ferraraccio, F., Cartenì, M., Riegler, G., de Magistris, L. & Fasano, A. 2011. Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: celiac disease and gluten sensitivity. *BMC Medicine*. 9: 23-34.

Schulze, M.B., Liu, S., Rimm, E.B., Manson, J.E., Willet, W.C. & Hu, F.B. 2004. Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women. *The American Journal of Clinical Nutrition*. 80: 348-356.

Van Tassel, D.L., DeHaan, L.R. & Cox, T.S. 2010. Missing domesticated plant forms: can artificial selection fill the gap? *Evolutionary Applications*. September. 3(5-6): 434–452.

Villeneuve, M.P., Lebeuf, Y., Gervais, R., Tremblay, G.F., Vuillemard, J.C., Fortin, J. & Chouinard, P.Y. 2013. Milk volatile organic compounds and fatty acid profile in cows fed timothy as hay, pasture, or silage. *Journal of Dairy science*. 96: 7181-7194.

Wade, N. 2006. *Before the Dawn: Recovering the Lost History of our Ancestors*. Penguin Books, NY.

Wells, S. 2010. *Pandora's Seed: Why the Hunter-Gatherer Holds the Key to Our Survival*. Random House, NY.

Wolter, A., Hager, A.S., Zannini, E. & Arendt, E.K. 2013. In vitro starch digestibility and predicted glycaemic indexes of buckwheat, oat, quinoa, sorghum, teff and commercial gluten-free bread. *Journal of Cereal Science*. 58: 431-0436.

Yang, M.S., Espinoza, N.O., Nagpala, P.G., Dodds, J.H., White, F.F., Schnorr, K. & Jaynes, M. 1989. Expression of a synthetic gene for improved protein quality in transformed potato plants. *Plant Science*. 64: 99-111.

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INTERCROPPING OF LEGUMES WITH CEREAL CROPS IN PARTICULAR WITH THE PERENNIALS TO ENHANCE FORAGE YIELDS AND QUALITY

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ABSTRACT:

Intercropping offers farmers the opportunity to engage nature's principle of diversity on their farms. Spatial arrangements of plants, planting rates, and maturity dates must be considered when planning intercrops. Intercrops can be more productive than growing pure stands. Many different intercrop systems are discussed, including mixed intercropping, strip cropping, and traditional intercropping arrangements. Pest management benefits can also be realized from intercropping due to increased diversity. Harvesting options for intercrops include hand harvest, machine harvest for on-farm feed, and animal harvest of the standing crop.

Since landholdings in Pakistan's mountainous Northern Areas are minute, farmers aim to maximize production per unit of area per season. An integrated approach that complements rather than competes with the existing farming system was needed. Forage production and availability have been affected by sole cropping vs. intercropping of forage legumes with cereals. To obtain early and good yields on small holdings under severe winter conditions, compatible fodder crops can be planted in mixtures to produce high fodder yields with good quality. Leguminous dwarf fodders like berseem can be mixed with taller species such as oats, ryegrass, brassicas etc. Lucerne is considered one of the most important leguminous fodder crops in Pakistan's Northern Areas.

Important priorities for future research include evaluating the potential for suitable cash cropping, promoting intercropping of potential fodder crops that might provide a more ensured/continuous supply over the winter, and improving the nutritional content of animal diets with, for example, the introduction/evaluation of improved alfalfa and fodder oats.

Keywords: intercropping, berseem, lucerne, alfalfa, fodder oats, soil fertility, sorghum

INTRODUCTION

Historically, intercropping has commonly been practiced throughout the developing world. Due to limited land holdings, farmers usually practice an integrated and subsistence type of farming system that is not very flexible. For example, in Africa, corn (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench), or millet (*Panicum* and *Pennisetum* spp.) are grown with pumpkin (*Cucurbita* spp.) cowpea (*Vigna unguiculata* (L.) Walp), pigeon pea (*Cajanus cajan* (L.) Millsp.), or beans (*Phaseolus* spp.). Cocoa (*Theobroma cacao* L.) is grown with yams (*Dioscorea* spp.) or cassava (*Manihot esculenta* Crantz). In the tropical Americas, maize (*Zea mays* L.) is grown with beans and squash (*Cucurbita* spp.). In both Africa and Latin America, beans or peas (*Pisum sativum* L.) climb tall cornstalks while pumpkins or squash cover the ground below. In these countries, many farmers have limited access to agricultural chemicals and equipment so prevalent in the developed world. Besides, intercropping is much less risky in that if one crop fails others may still be harvested (Machado, 2009).

Intercropping to reduce risk was a common practice in the United States and Europe before the 1940s, (Kass, 1978; Andersen, 2005), but the practice faded from significance as advances in mechanization and the availability of relatively cheap inorganic fertilizers and pesticides made monocropping more attractive. Paralleling the development of high-yielding varieties and production of cheap fertilizer that brought about the Green Revolution to feed rapidly growing populations, the practice of monocropping proved effective and economical (Horwith, 1985).

On the other hand, with fertilizer shortages developing and costs escalating, intercropping with legumes is again becoming desirable. The composite fertilizer price increased 113 percent between 2000 and 2007, led by gains in nitrogen prices (Huang, 2007). Meanwhile, environmental problems associated with heavy fertilizer use, e.g. surface- and groundwater pollution, soil acidification, and ammonia volatilization are becoming well known, and as synthetic fertilizer is a petroleum-based product, prices will continue to increase. Hence, fodder legumes such as alfalfa or lucerne (*Medicago sativa*), berseem (*Trifolium alexandrinum*), shaftal (*Trifolium resupinatum*), vetch (*Vicia sativa*), and cowpea (*Vigna unguiculata*) can be grown in association with fruit trees, providing fodder for livestock as well as improving soil fertility through biological nitrogen fixation. Oats might be a good choice for mixed planting with berseem or lucerne to maximize yields per unit area. There are several options available to enhance productivity through intercropping of several different crops. Some of these options are summarized below.

LEGUMES INTER-PLANTED IN ORCHARDS

The majority of the region's farmers rear livestock and also grow fruit trees. Therefore, an integrated approach that complements rather than competes with the existing farming system is required. In order to obtain superior quality fodder, improve soil fertility, and subsequently enhance fruit yields and quality, farmers intercrop lucerne, red clover, berseem, shaftal, or vetch in the orchards. Lucerne is considered one of the most important leguminous fodder crops that provide high quality hay for winter feeding.

Three improved winter active lucerne cultivars i.e. 'Sundar', 'Sequel', and 'Aquarius' were evaluated with a local cultivar in five to seven year old apple orchards in Chilas and Gilgit. 'Sundar' excelled over all cultivars in the double crop areas. With it, farmers have been able to harvest lucerne throughout the year on land protected from uncontrolled grazing.

Multicut forage sorghums (sorghum/Sudan grass hybrids), which were unknown in the area, provided an excellent means of increasing summer fodder production by producing three to four times as much fodder as the local maize. Local maize yields on average 39 tonnes/ha of green fodder, whereas the sorghum hybrid yields ranged from 110 to 138 tonnes/ha with an average of 127.7 tonnes/ha of air-dry material.

ENHANCED FODDER YIELDS, QUALITY AND SOIL FERTILITY PER UNIT AREA PER SEASON

In order to obtain early and good yields on small holdings in winter, compatible fodder crops may be sown in mixture to produce higher fodder yields and better quality per unit area per season. Short-statured leguminous fodders such as lucerne, berseem, and vetch can be mixed with oats, barley, ryegrass, brassica etc. Lucerne + oats, berseem + oats and shaftal +oats produced greater yields of green forage than did monocultures of the respective crops (Table 1).

TABLE 1. YIELDS OF SOME LEGUMINOUS FORAGE CROPS (TONNES/HA)

VARIETY	GREEN FORAGE	AIR DRY MATTER
LOCAL LUCERNE	55	19
SUNDER LUCERNE	120	45
SUNDER + OATS	190	58
SHAFTAL	45	14
SHAFTAL+OATS	80	25
BERSEEM	89	28
BERSEEM + OATS	130	47
OATS	90	38

Source: Dost, 1997

Oat + vetch and barley + vetch combinations produced 132-135 and 73-76 tonnes/ha of green fodder compared to 100 -105 and 56 -59 tonnes/ha pure oat and barley stands at both locations respectively in the 1 260 to 1 490 m altitude band (Table 2).

TABLE 2. GREEN AND DRY MATTER YIELDS (TONNES/HA) OF OATS, BARLEY, AND VETCH AT TWO SITES IN 1994-1997

CROPS	GILGIT (1490 m asl)		CHILAS (1260 m asl)	
	GREEN	DRY	GREEN	DRY
Oats	100	22	105	23
Oats + vetch	132	26	135	29
Barley	56	12	59	14
Barley + vetch	73	16	76	17

Source: Dost, 1997

A deep-rooted crop like lucerne can be intercropped with shallow-rooted crops like oats, rye, barley or a brassica; the annuals are usually sown between the rows of perennial fodder. Intercropping has a number of advantages over monocultures: more than one crop per season per unit area; easier weed control; higher yields than in pure sown crops; and fodder of better quality. Oats were intercropped in winter active lucerne and red clover in rows 30 cm apart at several sites. The intercropping of lucerne with oats produced greater green and DM yields than those of sole crops of either legume (Table 3).

Intercropping of oats with berseem clover provided earlier and greater fodder yields, and increased milk production by as much as 20 litres per cow per month on average compared with traditional practices. At the same time, the demand for purchased concentrates was reduced by 20 kg per month per animal and lactation period was extended by an extra two months (Dost, 1995).

TABLE 3. GREEN AND DRY MATTER YIELDS (TONNES/HA) OF LEGUMES AND OATS AT GILGIT IN 1993-1994

TREATMENT	GREEN YIELD	DRY MATTER
Lucerne	70	18
lucerne + Oats	115	30
Berseem	80	17
Berseem + Oats	135	30
Redclover	63	16
Redclover + oats	94	26

Source: Dost, 1995

Multiple cropping or mixed sowing techniques were carried out in North Pakistan by Dost (1997). The details are presented in Table 4.

TABLE 4. EFFECT OF MIXED SOWING ON GREEN AND DRY MATTER YIELDS (TONNES/HA) OF LUCERNE, RED CLOVER, AND OATS AT THREE SITES IN 1996-1997

TREATMENT	SULTANABAD		RAHIMABAD		SALING	
	GREEN	DRY	GREEN	DRY	GREEN	DRY
Lucerne cv. Sundar	110	30	105	26	68	20
Red clover	60	16	62	18	50	13
Oats	100	32	95	30	80	26
Lucerne + oats	140	39	136	37	102	30
Red clover + lucerne	115	32	105	26	70	22
Red clover + oats	90	26	93	28	75	23

Source: Dost. 1997

Oat has been used as a companion crop for sowing forages since the early 1990s in western Canada. In central Saskatchewan, oat was used at rates from 18 kg/ha to 72 kg/ha with 17 kg/ha sweet clover (*Melilotus officinalis*, *M. alba*) (Tinline, 1924). In southern Saskatchewan, Jefferson and Zentner (1994) sowed oats as a companion crop with lucerne on irrigated land. Lucerne sown alone produced much less than oat intercropped with lucerne or oat sown alone in the establishment year.

In Minnesota, Hartman and Sturtman (1983) recommended a seeding rate of 54-72 kg/ha for oat when used as a companion crop, compared with 72-90 kg/ha when sown alone for grain. Peter (1961) reported that oats cut for forage at the late dough stage plus a cut of intersown lucerne yielded more than lucerne established with or without herbicides and harvested twice in the establishment year. In contrast, Brink and Marten (1986) showed that oat as a companion crop to lucerne had inferior forage quality compared with barley when the mixture was harvested in the sowing year. In California, Lanini *et al.* (1999) reported that oat intersown into an established (but declining) lucerne stand was comparable to using paraquat herbicide for weed control, with the advantage of increasing first harvest forage yield. Marshal, McDaniel and Cregger (1992) suggested that growers planning to use oats as a companion crop should use early maturing, lodging-resistant cultivars, and remove the oat forage early to favour the establishing perennial forage crop.

NON WINTER-DORMANT VERSUS WINTER-DORMANT LUCERNE VARIETIES

One of the most important questions is whether we need perennials, winter-dormant or non-winter-dormant cultivars as they might affect the biomass as well as seed yields in the long run. Several perennial non-winter-dormant and winter-dormant varieties of lucerne were evaluated at three sites during 1993-1995. The details are presented in Table 5. Non-winter-dormant lucerne (Sundar being the main cultivar) has been extremely successful at all three sites. They may suffer some frost damage at high altitudes but grow throughout the year and yield more than twice as much as the winter dormant landrace in double-crop areas below 2 000 m. They also provided maximum green feed in the critical December-January period.

TABLE 5. GREEN AND DRY MATTER YIELDS (TONNES/HA) OF LUCERNE VARIETIES

VARIETIES	SITES					
	CHILAS		GILGIT		SKARDU	
	Green fodder	Dry matter	Green fodder	Dry matter	Green fodder	Dry matter
SUNDAR	165	50	174	52	90	26
MISASIRSA	98	32	117	34	74	20
PIONEER	92	29	95	30	86	22
SANORA	90	27	84	24	73	20
ILLUNICO	71	22	68	22	70	19
TYPE 8/9	100	32	96	30	74	21
POWERA	58	19	61	19	68	18
LOCAL	55	16	60	18	57	17
AVERAGE	91.13	28.38	94.38	28.62	73.25	20.38

Source: Dost, 1995

SINGLE CUT VERSUS MULTICUT FORAGE VARIETIES

Oat provides multiple cuts, tillers profusely, and yields more than wheat and barley in northern Pakistan. Standing oats can be cut progressively, releasing land earlier than normal for follow-on crops or relay cropping. Any remaining oats can be dried as hay. This coincides with optimum soil moisture for land cultivation and sowing of the following crop, and also allows small areas or peripheral lines on terraces to be saved for seed. In many, but not all instances, more recently bred cultivars outyielded older ones (Dost *et al*. 1994).

HYBRID VERSUS VARIETIES:

Local maize and millet are dual purpose crops that are extensively grown in North Pakistan. Multicut hybrid sorghum could ensure maximum tonnage of green as well as DM well distributed throughout the summer growing period.

Overall hybrid sorghums provided four cuttings in Gilgit and Chilas and two in Skardu and Khaiber. At all the locations, all hybrids produced two to three times more green fodder and DM yields well distributed over the entire growing period as traditional local maize and millet cultivars. Due to higher temperatures in Gilgit and Chilas, maximum forage yields were recorded as compared to Skardu and Khaiber.

CONCLUSIONS

It was observed that the multi-cut hybrid sorghums which were scarcely known in the area produced 100-125 tonnes/ha green fodder yields as compared to 25-30 tonnes/ha fodder yields by local maize. The improved varieties of maize were superior in grain, stover, and green fodder yields as compared to local landraces. However, improved varieties were 20 to 30 days late in grain maturity. Also the improved oats and lucerne varieties produced two to three times greater yields than local varieties.

The improved berseem clover varieties produced 132-140 tonnes/ha green fodder yields in six cuts as compared to 80-85 tonnes/ha by shaftal clover in three cuts. Although there is no tradition of applying chemical fertilizers to the forage crops in the region, maximum forage yields were obtained through application of 150-75 N-P kg/ha at most sites. However, increased use of fertilizer could not be justified in many instances for economic and environmental reasons.

REFERENCES

Andersen, M.K. 2005. *Competition and complementarity in annual intercrops—the role of plant available nutrients*. PhD thesis, Department of Soil Science, Royal Veterinary and Agricultural University, Copenhagen, Denmark. Samfundslitteraur Grafik, Frederiksberg, Copenhagen.

Brink, C. E. & Marten, G.C. 1986. Barley vs. oat companion crops. 1. Forage yield and quality response during alfalfa establishment. *Crop Science.* 26: 1060-1070.

Dost, M. 1995. *Quarterly report on fodder component*. UNDP/FAO project, Pak/86/027- Gilgit, Pakistan.

Dost, M. 1997. *Technical Reports of Fodder Components*, Pak/86/027- Gilgit, Pakistan.

Hartman, I. I., & Sturtman, D.D. 1983. A guide to quality and production. University of Minnesota Extension Bulletin (USA), No. AG-BU-209.

Horwith, B. 1985. A role for intercropping in modern agriculture. *Biological Sciences.* 35(5): 286-291.

Huang, W-Y. 2007. *Impact of Rising Natural Gas Prices on U.S. Ammonia Supply*. Outlook Report No. (WRS-0702). Washington, DC: USDA Economic Research Service.

Jefferson, P.G., & Zentner, R.P. 1994.Effect of an oat companion crop on irrigated alfalfa yield and economic returns in southwestern Saskatchewan. *Canadian Journal of Plant Science*. 74: 465-470.

Kass, D.C.L. 1978. Polyculture cropping systems: review and analysis. *Cornell International Agricultural Bulletin.* 32.

Lanini, W.T., Orloff, S.B., Bendixen, W.E., Canevari, W.M., Schmierer, J.L., & Vargas, R.N. 1999. Influence of oats (*Avena sativa*) interseeding on weed suppression in the final year of an alfalfa (*Medicag sativa*) stand. *Weed Technology.* 13: 399-403.

Machado, S. 2009. Does intercropping have a role in modern agriculture? J*ournal of Soil and Water Conservation.* 64(2).

Marshal, H.G., McDaniel, M.E., & Cregger, L.M. 1992. Cultural practices for growing oat in the United States. In H.G. Marshall and M.E. Sorrells (eds). *Oat science and technology,* Agronomy Monograph No. 33. Madison, WI, USA, Crop Science Society of America.

Peter, R.A. 1961. Legume establishment as related to the presence or absence of an oat companion crop. *Agronomy Journal.* 53: 195-198.

Tinline, M.J. 1924. *Hay and pasture crops in Northwest Sashatchewan, Dominion of Canada Department of Agriculture*, Ottawa, Canada. Bulletin 40, New Series.

17

DEVELOPMENT OF CONTINUOUS LIVING COVER BREEDING PROGRAMMES TO ENHANCE AGRICULTURE'S CONTRIBUTION TO ECOSYSTEM SERVICES

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ABSTRACT

Over the last half century, 'Green Revolution' technologies have dramatically enhanced crop yields, but because of the emphasis on annual row cropping systems these increases have often come at the expense of food security and sustainability. Globally, many fear that agriculture is nearing a tipping point, with concerns that population pressure, declining natural capital, and diminished ecosystem service delivery will reduce global food security. As a result, a new Green Revolution is needed – a ‘Forever Green Revolution’ – that embraces continuous living cover on working lands through the development of a new suite of high yielding perennial (intermediate wheatgrass, sunflower, hazelnuts) and winter annual (pennycress, winter rye, winter barley) crops that provide economic return and improve multiple ecosystem services. By adding such crops to agricultural systems we can: enhance agricultural productivity, support rural economic development, and provide major environmental benefits to all citizens. Because these systems have longer growing seasons, they are able to capture more solar energy, water, and nutrients than purely annual systems and may be able raise crop yields, produce new high-value commodities (food, feed, and biomaterials), enhance soil quality, provide wildlife habitat, increase species biodiversity, and improve water resources. Additionally, agricultural systems that include perennial and winter annual crops may show greater resilience to climate change, as well as to weed, disease, and insect pressures. To accomplish a ‘Forever Green’ landscape, we propose three significant shifts in thinking: 1) focus public plant breeding programmes on development of crops that provide continuous living cover and high-value commodities 2) diversify and enhance agricultural stakeholder engagement in sustainable enterprise development, and 3) re-evaluation of concepts of production and efficiency in agricultural systems.

Keywords: agro-ecosystem, economic valuation, ecosystem service, RUSLE, tradeoff analysis yield

INTRODUCTION

Over the past half century, Green Revolution technologies have dramatically enhanced crop yields (Baulcombe *et al.* 2009) while simultaneously reducing other ecosystem service outputs (Tilman *et al.* 2002). Globally, many fear we are nearing a tipping point (Garnett *et al.* 2013), and that given increased population pressure (Foley *et al.* 2011; Runge *et al.* 2003), declining natural capital (Jordan *et al.* 2007), and overall diminished ecosystem service delivery (Tilman *et al.* 2011) a new green revolution is needed – a “Forever Green Revolution” – that embraces continuous living cover on working lands through a new suite of perennial grain and biomass crops, and winter annual crops. Conceptually, this is related to the concept of evergreen agriculture that has been discussed as a way to improve food security across the world (Garrity *et al.* 2010). These crops must not only enhance profit for landowners, commodity groups, and agribusiness, but also ecosystem services for society. We propose that a sustained focus on developing continuous living cover is an essential avenue for sustainable intensification of agriculture (Garnett *et al.* 2013).

The potential benefits of continuous living cover have long been touted (Teasdale *et al.* 2007, Scheinost *et al.* 2001): decreased autumn tillage during the multi-year lifetime of a crop stand, leading to reduced input costs and soil erosion; reduced herbicides from spring weed suppression; increased habitat for beneficial insects (pollinators and predators), providing a biological control that reduces inputs and increases pollination services; decreased surface and subsurface water pollution. However, despite these benefits, relatively little has been done to include these crops in rotations or improve them. Nationally, perennial grains and winter-cover crops constitute less than 7 percent of all cropland (Wallander, 2013) in the United States. This is likely due to the limitations of current plant material to improve environmental quality and simultaneously increase economic viability of agricultural operations. However, it is possible to breed with multiple benefits in mind – benefits not only including high yield, but also increased ecosystem service delivery. The starting and ending point of sustainable intensification is land management, which primarily focuses on the questions, "What plant material is available?" and "Where should plant material be placed on the landscape?" To implement continuous living cover in current temperate-zone agro-ecosystems, there are two major options: winter-hardy annuals, and perennial grain and biomass crops.

Historically, winter-annual crops have provided multiple values to landowners not just as a winter cover, but also as livestock feed. Today, the increased segregation of animal and plant agricultures (Godfray *et al.* 2010) and the shift in animal rations toward maize and soybean derivatives, has meant that winter and cover crops are no longer as relevant to producers. Forage legumes and grasses are still important parts of the landscape, but they are disappearing due to this increased separation between animal and crop agriculture. While current winter annual cover crops such as winter rye (*Secale cereale* L.) can mitigate the off-site nutrient transport, soil erosion, and loss of soil organic matter that occurs under a maize (*Zea mays* L.) and soybean (*Glycine max* L.) rotation without jeopardizing landowners' livelihoods (Creamer *et al.* 1996; Strock *et al.* 2004; Kaspar *et al.* 2012), they offer little other value to farmers. Additionally, farmers commonly find current cover crop options difficult to establish and terminate without increasing risk to the subsequent cash crop (Leavitt *et al.* 2011). These concerns largely explain the small area devoted to cover crops in the United States. In response to cover crops' lack of economic viability, new winter annuals are being evaluated and developed, such as pennycress (*Thlaspi arvense*) and camelina (*Camelina sativa*). Both produce valuable oilseed in addition to their other ecological benefits (Phippen and Phippen, 2012).

The second form of continuous living cover is perennial grains and other herbaceous perennial crops, including high-yielding biomass crops. Perennial grains are less well-developed than other perennial crops; initial attempts to produce a perennial grain have been met with mixed results, with yields ranging from 10-70 percent of annual check cultivars (Scheinost *et al.* 2001; Sacks *et al.* 2003; Sacks *et al.* 2006). These mixed results have led some to question whether it is possible to breed a high-yielding perennial grain. This debate centres on whether it is physiologically possible for a plant to allocate resources to both sexual and asexual production in a way that would allow for yields comparable to sexual grains. Additionally, it has been argued that high yielding perennial grains do not occur in nature, have not already been domesticated, and therefore, are likely impossible to develop.

Counter to this, perennial plants introduced to novel environments where consumers are absent can experience rapid evolutionary change and allocation of resources to increased seed and biomass production (Evolution of Increased Competitive Ability Hypothesis) (Bossdorf *et al.* 2005). Further, Cox *et al.* (2002) and DeHaan *et al.* (2005) developed a framework suggesting that because of a longer growing season, perennial grains could capture more sunlight resources resulting in greater total biomass, which could be allocated to seed production. Indeed, many of the arguments against high-yielding perennial grains have drawn information from what is possible or observed in natural systems. But, in the novel environment of an agricultural system, it may be possible to develop new life strategies by changing the selective constraints the plants experience. For instance, insect herbivory, soil nutrients, water availability, and the degree of group selection all can be varied in an agricultural system. Barnes *et al.* (2013) explored some of these possibilities by developing a physiologic model of plant resource allocation that showed perennial seed production equaled or surpassed that of annuals under certain conditions, implying that high-yielding perennial grains may be bred for in the real world, and may offer a competitive alternative to annuals. Additionally, Bell *et al.* (2008) has shown that, under certain conditions, even if a perennial grain crop produces 30 percent less yield than an annual system, decreased input costs can make up the difference in profit, even as the perennial crop provides additional ecosystem services.

Major questions remain regarding perennial grains such as how they will respond to domestication. Will perennial grains transition similarly as annual crops and undergo "domestication syndrome" (the development of a series of traits related to domestication, which have been altered in a similar way in many species across many taxa) (Harlan, 1992; Vaughn *et al.* 2007; Weeden, 2007)? Even more uncertain is whether the annual domestication syndrome phenotype is the ideal phenotype (ideotype) for a perennial grain domesticate. For example, does the ideotype of the perennial *Helianthus* seed crop have a single inflorescence or multiple inflorescences that flower simultaneously (Kantar *et al.* 2014)? The above findings and questions simultaneously reinforce the need for continued research investment in perennial grains and provide cautious hope surrounding their potential success.

In order to rapidly develop continuous living cover as a strategy for sustainable intensification of temperate-zone agro-ecosystems, we call for interrelated paradigm shifts in two areas – plant breeding and stakeholder engagement. In essence, we argue that breeding must be situated in an integrative and systemic approach to sustainable intensification. Below, we describe a new approach to development of plant germplasm for sustainable intensification of agriculture. We term this approach the '*Reflective Plant Breeding Paradigm*' and we are developing it in the context of an ongoing research and development programme for continuous living cover and sustainable intensification at the University of Minnesota.

THE FOREVER GREEN INITIATIVE

The Forever Green initiative lays out a cohesive vision for how to accomplish "sustainable intensification" of the Upper Midwest agro-ecosystem. The initiative grew out of Minnesota's history with cover crops and perennial grains as well as the obligation of a Land Grant University to engage with multiple stakeholders: farmers and their advisors, agricultural industry, and the general public. Realizing this obligation resulted in the merging of traditional plant breeding focused on farmer needs with a diverse array of disciplines (Table 1). We are approaching this task from the ideological point of view that germplasm must be developed to create both economically and ecologically profitable crops. The initiative involves more than 15 disciplines ranging from ecology and agronomy to plant breeding and food science to economics and sociology, all focused on two interconnected questions: 1) What plant material? and 2) Where is the material best placed on the landscape? These two questions form two continuous, synergistic feedback loops where enterprise development and stakeholder engagement interact with the plant breeding process in the Reflective Plant Breeding Paradigm (Figure 1). The Reflective Plant Breeding Paradigm includes robust engagement of many different disciplines in order to define the agro-ecological performance of germplasm, and define the trade-offs and synergies that are present as part of the germplasm being tested under different enterprise development scenarios (Figure 1). The 'Forever Green' initiative is an attempt to empirically develop crops that when strategically placed on the landscape will fit new ecological niches and provide environmental services while simultaneously providing economic benefits through a commercial product. In essence, it is an empirical attempt to test "sustainable intensification". Specifically, the 'Forever Green' initiative is examining a wide range of crops including winter-annuals, short-rotation woody species, perennial grains, and perennial plants for natural products (individual projects are outlined in Table 1).

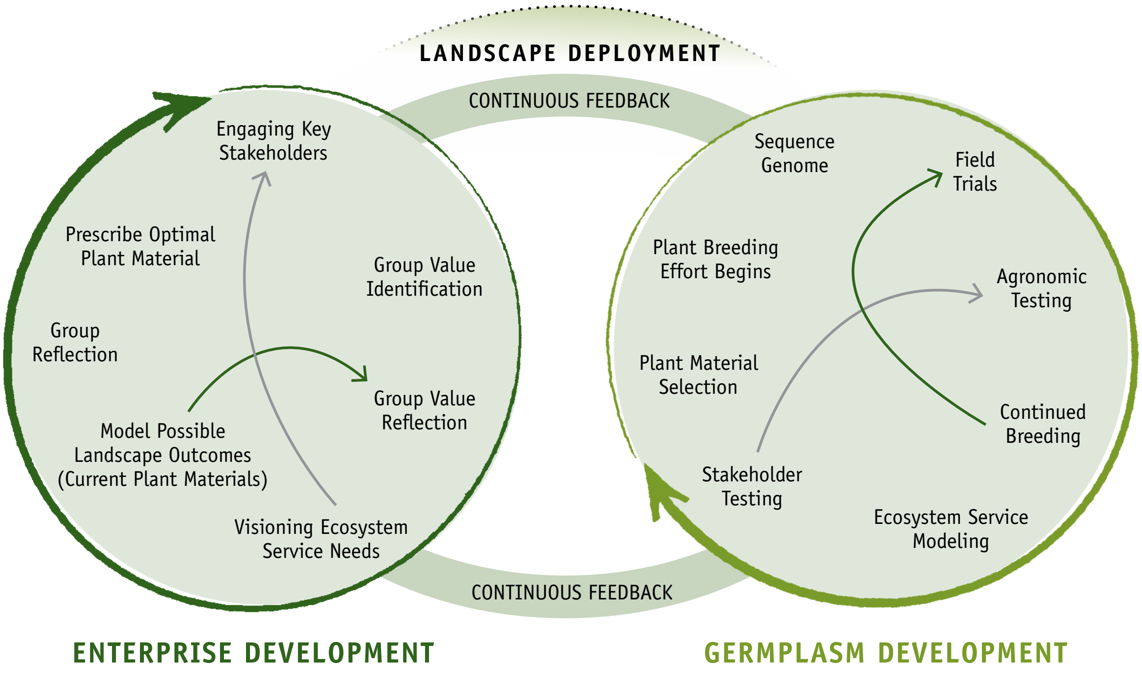
TABLE 1. BRIEF DESCRIPTION OF SOME OF THE CROPS THAT THE UNIVERSITY OF MINNESOTA IS WORKING ON TO INCREASE YEAR-ROUND GROUND COVER

CROP	DESCRIPTION OF PROGRAM	UNIVERSITY OF MINNESOTA DEPARTMENTS INVOLVED
INTERMEDIATE WHEATGRASS	A perennial grass crop that can produce many different high-value products, providing economic opportunities that in turn support the environmental benefits that perennials provide. It produces large yields of seeds that are a high-quality substitute for wheat, while its dense root system and rapid regrowth after harvest build soil carbon, store water for later use, and prevent soil erosion. It can also be harvested for hay or biofuel and is highly tolerant of weather extremes, including droughts and intense storms.	Agronomy and Plant Genetics Applied Economics Soil, Water, and Climate Ecology, Evolution, and Behaviour Food Science and Nutrition Plant Pathology Public Policy

CROP	DESCRIPTION OF PROGRAM	UNIVERSITY OF MINNESOTA DEPARTMENTS INVOLVED
FIELD PENNYCRESS	A new winter-annual cover crop for corn/soybean farmers. It is planted after harvest of maize or soybean and resumes growth in early spring after winter dormancy. It provides crucial protection for soil during autumn, winter and spring, and produces high-value oil and protein meal from unused fertilizer and water that would otherwise be wasted. As well, pennycress suppresses weed growth, reducing herbicide costs, and supports honeybees and other endangered pollinators.	Agronomy and Plant Genetics Applied Economics Soil, Water, and Climate Ecology, Evolution, and Behaviour Plant Biology Plant Pathology Bioproducts and Biosystems Engineering Animal Science
WINTER MALTING BARLEY	A potentially high value cover crop that could be double cropped with soybeans. Current winter barley varieties do not consistently survive winters in northern climates.	Agronomy and Plant Genetics Animal Science Plant Pathology
WINTER CEREAL RYE	A winter-annual cover crop that has been shown to provide many environmental services without impacting the soybean yields in a corn/rye/soybean rotation.	Agronomy and Plant Genetics
PERENNIAL FLAXSEED	An excellent source of omega-3 fatty acids, whose value as a dietary supplement is widely recognized, while offering the soil protection, habitat, and resource-use benefits of perennial crops. An emerging natural products industry is interested in sourcing key ingredients for many products from native and sustainably-grown crops.	Agronomy and Plant Genetics
PERENNIAL SUNFLOWER	An emerging perennial crop that can produce food oils that are highly valuable because they are free of trans fats, while also providing all of the benefits of perennial crops, including use of otherwise-wasted resources, soil protection, reduced costs, and better tolerance of droughts and floods, which are predicted to become more common in coming years.	Agronomy and Plant Genetics Ecology, Evolution, and Behaviour Horticulture Law School Entomology American Indian Studies
HAZELNUTS	A new food and energy crop. Hybrids between native and European hazelnuts combine beneficial qualities of each. As a long-lived shrub, hazelnuts can fit profitably into many niches in the agricultural landscape. For example, farmers could gain significant revenue from hazelnuts grown as windbreaks, shelterbelts, and living snow fences. In addition to valuable nuts, mature hazelnuts can produce large yields of edible, heart-healthy oils or biofuel oils.	Agronomy and Plant Genetics Horticulture Plant Biology Forestry
WILLOWS	A rapidly growing woody perennial crop. As a small tree, this crop can provide many options for improving the habitat value of Minnesota landscapes, while providing all of the advantages of perennial crops and providing new bio-products, including sustainably produced construction materials and bioenergy. Grown and harvested on a three-to-five-year cycle, willows can bring substantial revenue streams to farms that can support the environmental benefits that they provide.	Agronomy and Plant Genetics Horticulture Plant Biology Forestry
ALDERS	Woody trees and shrubs with the capacity to be grown on sites that cannot support traditional row-crop agriculture. Due to the symbiotic relationship alders form with the nitrogen-fixing bacterium Frankiia alni, the trees can be gown on low-nutrient soils without the need for additional nitrogen inputs. The species naturally occur on wet margins and saturated soils, areas that are not typically farmed. As such, alders represent a potential bioenergy crop that will not compete with food crops for growing space on the landscape.	Agronomy and Plant Genetics Horticulture Plant Biology Forestry
KURA CLOVER	A crop with unique application in soil conservation and as a living mulch crop. We propose to promote use of Kura clover as a living but suppressed perennial sod into which maize or other grain crops are planted into strips killed with an herbicide. When the crop is harvested, Kura clover, which has underground- spreading rhizomes, can regrow into the space where the maize was grown. The Kura clover can then be grazed in the late autumn and following year.	Agronomy and Plant Genetics Horticulture Plant Biology Soil, Water, and Climate
NATIVE PERENNIAL SPECIES	Native species across the Upper Mississippi River Basin have been examined for antimicrobial, antifungal, and antioxidant activity. Promising species have been examined and selected for larger-scale production.	Agronomy and Plant Genetics Horticulture Plant Biology Law School American Indian Studies

FIGURE 1. SHOWS SYNERGISTIC RELATIONSHIP AMONG STAKEHOLDER ENGAGEMENT, BREEDING, AGRONOMICS, AND MODELING THAT ARE A PART OF THE FOREVER GREEN INITIATIVE'S ATTEMPT TO DEVELOP A REFLECTIVE PLANT-BREEDING PARADIGM

The illustration shows Enterprise Development focusing on stakeholder engagement on the left, and research goals focusing on Germplasm Development on the right, with a permeable membrane connecting the programmes. Lines within each programme indicate an example of feedback relationships among the various parts; in theory all nodes are interconnected. The Reflective Plant-Breeding Paradigm is built upon the traditional plant-breeding paradigm, which is primarily focused on enhancing crop yield and disease resistance. The new paradigm attempts to bring the traditional strengths of plant breeding into contact with other disciplines such as ecology and public policy in order to effectively identify and select plant material and characteristics that will maintain yield and simultaneously provide the greatest number of other environmental services that are required for a truly sustainable system. The University of Minnesota's programme incorporates perspectives from 15 different disciplines to more effectively address the challenges of new crop and enterprise development, while acknowledging that plant material will only be adopted across the landscape if it is economically profitable. The integrative approach helps identify the appropriate plant material, landscape position, and end use for a plethora of plant material.



The Forever Green initiative is engaging with enterprise development and stakeholders as part of the plant breeding process to answer the "what" and "where" from communities' perspectives. Research in social learning shows that often people react in unexpected ways to newly developed scenarios depending on their perspective (Johnson *et al.* 2012). By including the public in discussions through social learning processes, there is a greater sense of ownership where potential social and scientific solutions can be more easily understood and imagined

(Johnson *et al.* 2012). Recent calls for more system-based approaches (Power, 2010) require that the public perception of new technologies be included in any assessment of their potential use. Specifically, landowners need to be shown the direct benefits of new plant materials for any changes in management practices to occur. Ultimately, short of heavy-handed legislation, landowners will be the ones to implement land cover change, so it is critical to include their input and values in the design of new plant material. In addition, it is essential that the process be transparent, equitable, and inclusive of all stakeholders in land management.

Many landowners perceive that one or more high-yielding crops mean the most profits. Our goal is to change that perception to one that is more holistic, so that landowners are concerned with net economic output (including ecosystem services) per hectare over time. This could incentivize double cropping and justify the potential yield reduction of one crop in favour of new practices that could increase the overall profitability of and reduce the risk to the agricultural system. This would require a dramatic culture shift among farm communities. As Warner (2007) stated, “the greatest obstacle to ecologically informed alternative practices has not been a shortage of ideas; it has been the dearth of practical educational initiatives.” The process would need to be conducted iteratively over a long period of time to allow social learning to take place (Dana and Nelson, 2012).

Accordingly, a pivotal feature of the Reflective Plant Breeding Paradigm is the developing concept of ‘*Landlabs*’ (Jordan *et al.* 2013). These are place-based, coordinated efforts to design and implement new agricultural enterprises that meet high performance standards in economic, environmental and social terms. Landlabs engage a wide range of local and regional stakeholders and innovators. The goal is to engage these actors to develop and coordinate novel land-use configurations, supply chains, and policies necessary for the emergence of new sustainable enterprises. In essence, Landlabs serve as active “incubators” for coordinating technological, economic and policy innovations in enterprise development, and thereby reduce the economic and environmental risks and uncertainties faced by farmers, entrepreneurs, and public and private investors. Thus, Landlabs provide a social and institutional context for the coupling of germplasm and enterprise development (Figure 1) that is essential to the Reflective Plant-breeding Paradigm.

Simultaneously, multiple academic disciplines are working together to respond to the findings in the Landlabs to further refine the genetic resources required by the public. This is being done through an iterative process of breeding and then modelling landscape scale performance. These findings are being provided on an ongoing basis in Landlabs to inform the innovation needed for sustainable enterprise development (Jordan *et al.* 2013). Ideally, the Reflective Plant Breeding Paradigm will engage stakeholders by identifying new plant material that fits changing values and production needs. Farmers will then play an integral role in testing new material and providing feedback to make sure that the shifting target of “sustainable intensification” is met without compromising the values of people or the researchers. The process involves iterative stages allowing for simultaneous enterprise and germplasm development (Figure 1). Incorporating a process of value identification and testing into germplasm development may facilitate adoption once the material is developed (Jordan *et al.* 2011).

New production systems that combine summer annual crops, winter annual crops, and perennials can optimize use of limited land, water, and nutrient resources more efficiently than current systems do. For this reason, we call these systems high-efficiency agriculture. These systems are arguably the most promising vehicle by which we can rapidly and sustainably intensify agriculture and enhance its ability to withstand climate variability. In a spirit similar to that of the Reflective Plant-Breeding Paradigm, two areas need further research and development to realize the great potential of these high-efficiency systems: 1) genetic improvement of plant materials, and 2) development of new strategies to integrate perennial crops into the landscape in ways that provide environmental benefits and economic opportunities. Current work at the University of Minnesota on high-efficiency agriculture systems—as part of the Forever Green initiative—focuses on a portfolio of highly promising options for improving Minnesota agriculture's productivity, efficiency, and adaptability to variable climates (Table 1). Although each individual programme has its own unique challenges, all are being evaluated based on the Reflective Plant-Breeding Paradigm (Figure 1). The Forever Green initiative represents an empirical attempt to put into practice the theory of sustainable intensification whereby systems are created that can successfully increase ecosystem service delivery and economic profitability.

ANALYSIS OF TRADEOFFS AND SYNERGIES AS THE LENS OF SUSTAINABLE INTENSIFICATION

While the Reflective Plant-Breeding Paradigm encompasses both enterprise and germplasm development, germplasm development and landscape deployment are both explicitly and implicitly involved in economic and ecological tradeoffs and synergies. Analysis of tradeoffs at the plant and landscape scale frames the process of enquiry in terms of what is biophysically and politically possible. At the plant scale, we are actively working to explain and model the tradeoffs between length of life and annual seed production. Theoretically, it is possible for a perennial to be high yielding (Barnes *et al.* 2013), however several potential constraints merit further consideration. In *Helianthus*, for example, the advancement of yield is being weighed against with other essential agronomic characteristics such as synchronous flowering time and shattering. In intermediate wheatgrass, the interaction between nutrient treatments and baking quality and post-harvest processing is being examined. In pennycress, the interaction between yield of the cover and yield of the subsequent soybean crop is being investigated. Emerging results suggest that old and new breeding techniques can either entirely overcome the initial tradeoffs or significantly mitigate their severity in many cases.

Implementation of perennial crops at the landscape scale suggests several areas where synergies or tradeoffs occur among ecosystem services. Four major ecosystem services – sediment retention, carbon sequestration, pollinator services, and biological control – are examined qualitatively below. First, it has long been observed that an increased reliance on the corn-soybean rotation has led to increased sediment and nutrient loss with small critical landscape positions contributing disproportionally more sediment and phosphorous to waterways (Galzki *et al.* 2011). However, implementing current best management practices, which do not target landscape positions for conservation practices, would lead to only incremental reductions in nutrient export (Vache *et al.* 2002). Identifying fine scale differences in terrain could allow for better temporal and landscape position of management practices to ensure maximum conservation benefits (Galzki *et al.* 2011). Further, nutrient and sediment loadings in waterways can have significant adverse effects on humans and ecosystems (Jones *et al.* 2001). Strategic development and landscape placement of new perennial plant material could lead to disproportionately large reductions in sedimentation at the watershed scale (Parish *et al.* 2012) while producing economically competitive yields, an example of synergism among ecosystem services and agricultural productivity.

Second, increasing soil carbon is an important ecosystem service to mitigate climate change and can be accomplished by land use changes (Powlson *et al.* 2011). Recently it has been shown that reductions in carbon emissions from reduced tillage are not as large as previously thought (Luo *et al.* 2010; Mishra *et al.* 2010), however the reductions from changing annual vegetation to perennial vegetation still have the potential to decrease atmospheric CO_2 (Collins *et al.* 2010). Therefore, perennial crops can potentially increase the amount of carbon that is sequestered in stable forms in agricultural soils.

Third, another significant benefit of continuous living cover cropping systems is their potential to attract and support beneficial insects for pollination and biological control. For example, there is widespread evidence showing that diversification of cropping systems enhances biological control of insect pests (Letourneau *et al.* 2011). Provisioning resources, such as floral nectar and pollen, in a diversified planting can attract and enhance predator populations leading to greater biological control (Hogg *et al.* 2011). Particular perennial plants and plant breeding programmes have the potential to contribute germplasm that enhances biological control. For example, *Helianthus* species are known for producing extra-floral nectaries, a nectar source excreted primarily from the petioles. Such nectar can provide an early pre-flowering, alternative resource for such beneficial predators as coccinellid beetles, which are shown to perform equally on sugar versus prey-only diets (Lundgren, 2009). Further, sunflowers have been shown to increase the density of these beetles in adjacent annual crops (Jones and Gillett, 2005). Given the importance of coccinellids as a beneficial predator (Gardiner *et al.* 2009), there is potential for strategic integration of perennial sunflowers to enhance biological control. This further illustrates the value of breeding for multiple benefits including nectar production for biological control while producing seed for oil production.

There are many and varied perspectives on what is considered highly productive. Productivity is intertwined with cultural values and, in practice, incorporation of values is accomplished through stakeholder engagement. Tradeoffs and synergies between ecosystem service phenotypes and traditional phenotypes for breeding programmes provide new targets for plant breeders; these phenotypes are inherently based on a different scale than traditional measures of productivity. These considerations are necessary to define the set of ecosystem goods and services that are valued by stakeholders in any given situation, and to define goals for breeding in the context of developing new sustainable agricultural enterprises.

A CASE STUDY: WATONWAN COUNTY, MINNESOTA

To demonstrate how new plant material could potentially function in a highly productive region of the United States, we conducted a case study involving the fertile landscape of southern Minnesota. Specifically, our analysis of Watonwan County, Minnesota, illustrates the *Germplasm D*evelopment side of the Reflective Plant-Breeding Paradigm, where breeding, agronomic testing, and continued breeding feed into ecosystem service modelling (Figure 1).

Minnesota has 10.93 million hectares of farmland, occupying nearly half the 22.5 million hectares in the state. Two highly productive and profitable crops, maize (3.52 million hectares planted in Minnesota in 2012) and soybean (2.87 million hectares) are the foundation of the state's agriculture, together with other important production systems such as animal agriculture, small grains, and horticultural crops. Most of Minnesota's current cropping systems consist of summer annuals. Considering Minnesota's strong cropping system base and the in-development plant materials of pennycress and intermediate wheatgrass, we began to ask: How do current Minnesota agro-ecosystems compare with the native prairie ecosystem in terms of ecosystem service delivery? How will these new crops potentially alter the delivery of ecosystem services when compared with current cropping systems and the native prairie?

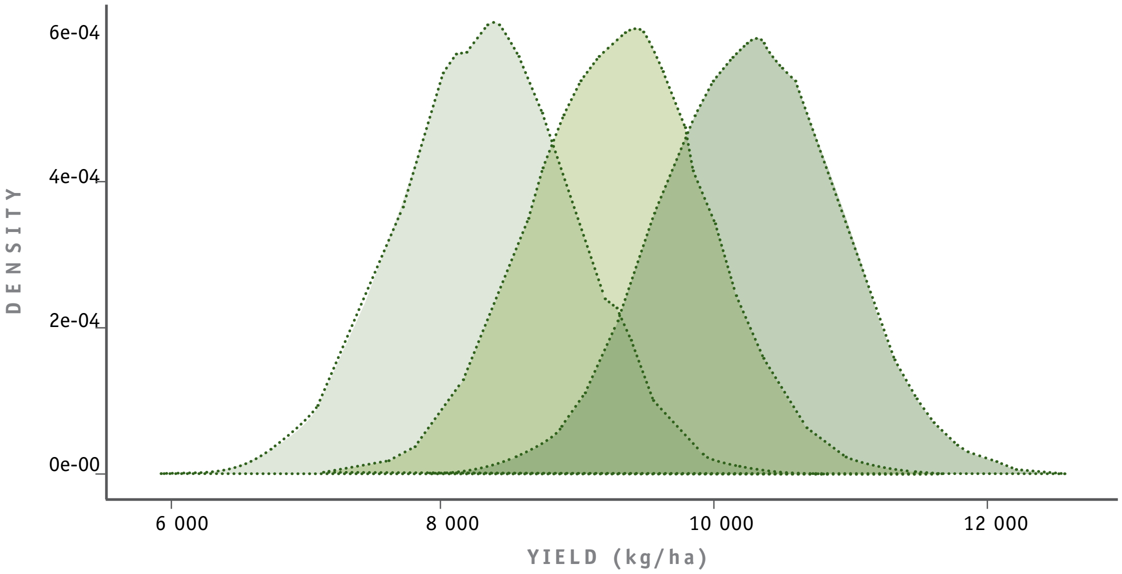
We performed a preliminary analysis that examined the tradeoff between the ecosystem services of sediment retention and total net return in the county given seven crop rotations – continuous maize (C), continuous soy (S), maize/soybean (CS), maize/rye/soybean (CRS), maize/pennycress/soybean (CPS), soybean/spring wheat (SW), and continuous intermediate wheatgrass (IWG). *We hypothesized that the new crops would enhance sediment retention and net economic output of Watonwan County, when compared with currently existing cropping practices.*

EXPERIMENTAL PROCEDURE FOR WATONWAN COUNTY, MINNESOTA CASE STUDY

For a full description of methods see Appendix 1. To summarize, we modelled sediment retention with the Revised Universal Soil Loss Equation (RUSLE) altered slightly to be applied in a geographic information system (GIS). We downloaded the baseline digital elevation model of Watonwan County from the Minnesota Department of Natural Resource's data warehouse. The 2006 National Land Cover Dataset (NLCD) for Watonwan County was downloaded from the Multi-Resolution Land Characteristic Consortium website (Fry *et al.* 2011) in order to differentiate between cropped and non-cropped land. To calculate the net economic return for each cropping system over the period of 2001-2010, we utilized crop production economic data containing average yield, production costs, gross return per acre (converted to gross return per hectare), net return per acre (converted to gross return per hectare, and price per bushel from the USDA-Economic Research Service (ERS) (retrieved July 2013).

Yield for each crop within each grid cell (100 m^2) was determined by creating three random normal distributions – a high, average, and low (Figure 2). Crop yield for each grid cell was then multiplied by the average value of the crop over a ten year period, and then adjusted to represent the respective value in rotation with other crops. To explore the comparative delivery of ecosystem services offered from the different cropping systems compared with the native prairie, we developed a series of landscape change scenarios. The scenarios consisted of transitioning the cropped area of Watonwan County from 100 percent native prairie to 100 percent agro-ecosystem for each crop rotation listed above. Land was placed into a rotation in 10 percent increments by soil erosion decile. Soil erosion and net return were summed across the landscape for each cropping system scenario. Graphs were all created using ggplot2 (Wickham, 2009) in R version 3.0.1 (R Core Development Team, 2013).

FIGURE 2. CORN-YIELD DISTRIBUTIONS CREATED AT RANDOM FROM EMPIRICAL DATA, USED TO MODEL YIELD ACROSS THE LANDSCAPE

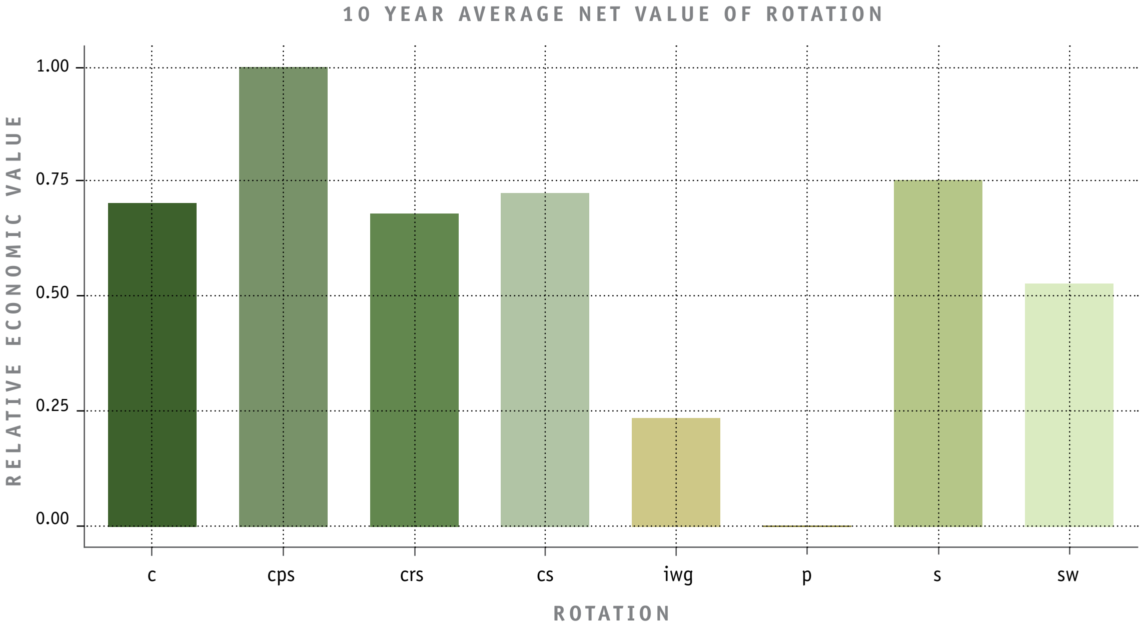


CASE STUDY RESULTS AND DISCUSSION

By modelling the effect of rotations on the potential for soil loss in Watonwan County; with RUSLE, we observed several trends. The seven rotations examined over a ten year time frame resulted in the following projections for soil-loss risk per crop rotation, ordered from greatest to least: SW, C, CS, S, CPS, CRS, IWG, and P (native prairie) (Figure 3). Comparatively, the C, S, and CS rotations, and the CRS and CPS rotations were similar. Intermediate wheatgrass had the least soil erosion potential compared with the other cropping systems analysed. SW had the greatest erosive potential likely due to the short amount of time spring wheat covers the landscape. P had essentially no erosion, which is verified in the literature (Kort *et al.* 1998). In Watonwan County ~15 percent of the land is at risk for sediment loss (greater than 5.5 Mg/ha/yr potential soil loss), so while the county in general is not at risk certain landscapes are, and different rotations could be used on these landscapes. For example, an intermediate wheatgrass planting reduced the risk of soil loss by approximately threefold compared with a corn/soybean rotation (Figure 3). Our data suggest that there is a great benefit from going to continuous cover on any landscape position; however, the greatest benefit will be seen on marginal lands.

FIGURE 3. RELATIVE SEDIMENT LOSS RISK DERIVED FROM THE REVISED UNIVERSAL SOIL LOSS EQUATION (RUSLE) OVER A 10-YEAR CROPPING SYSTEM OF EIGHT DIFFERENT CROPPING ROTATIONS

C = continuous corn, CPS = corn/pennycress/soybean, CRS = corn/rye/soybean, CS = corn/soybean, P = prairie, IWG = intermediate wheatgrass, S = continuous soybean, SW = soybean/wheat.



The modelled economic return from greatest to least was CPS, S, CS, C, CRS, SW, IWG, and P (Figure 4). Native prairie (P) was valued at zero because our interest was in comparing an unmanaged ecosystem to an agro-ecosystem, though we acknowledge that prairie mixtures could potentially be harvested and sold for biomass. Over the ten-year period, C, S, CS, and CRS produced similar net economic returns. If we had done the analysis over a shorter time period – say from 2008 to 2012 – we would have likely seen different economic outcomes because of the high value of maize and soy starting in 2008 caused partially by the United States' ethanol mandate (Zilberman *et al.* 2013) and an increased demand for soybean as animal feed in China (Godfray *et al.* 2010). The IWG rotation performed at approximately a third of the value of the CS rotation. The CPS rotation produced the greatest net return economically. This likely resulted from the ability of the CPS rotation to capture the high productivity and value of the CS rotation while simultaneously adding an additional cash crop half of the years, whereas rye does not offer the same economic benefits. Our analysis corresponded with USDA-ERS national average data for the general economic trends where data was available (Figure 5).

FIGURE 4. RELATIVE TOTAL NET ECONOMIC RETURN FOR A 10-YEAR CROPPING SYSTEM OF SEVEN DIFFERENT CROP ROTATIONS

C = continuous corn, CPS = corn/pennycress/soybean, CRS = corn/rye/soybean, CS = corn/soybean, P = prairie, IWG = intermediate wheatgrass, S = continuous soybean, SW = soybean/wheat.

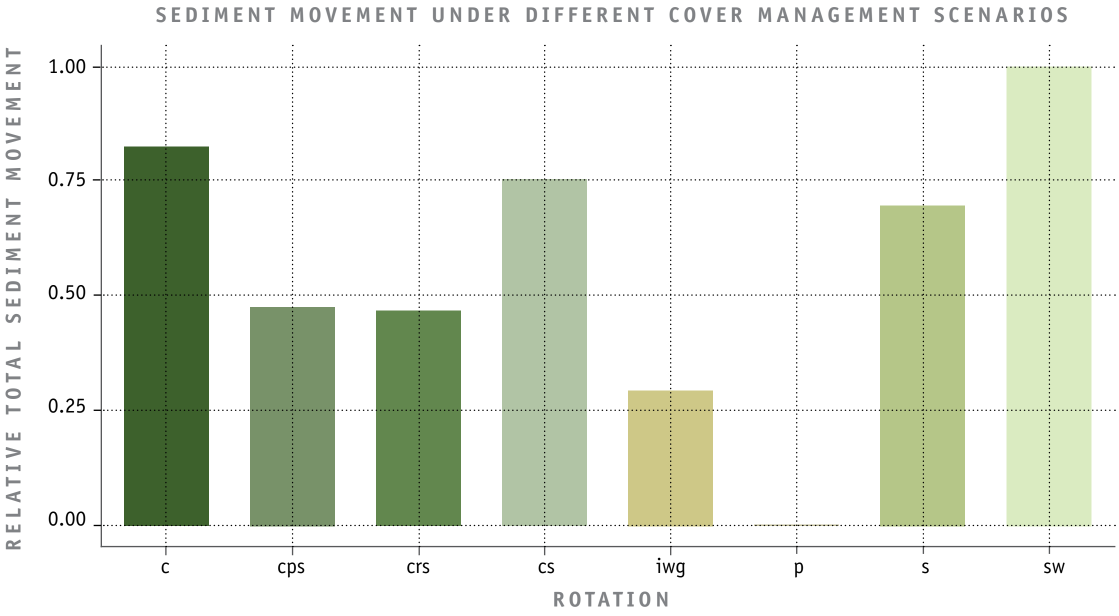


FIGURE 5. TEN-YEAR MEAN FOR NET RETURN PER HECTARE FROM USDA-ERS, 2001-2010

Intermediate wheatgrass return was calculated by discounting the value to 85 percent of wheat and modifying the input costs to account for decreased seed and field pass cost. Pennycress value was calculated as 50 percent of the value of soybean with the input costs being discounted, as it is only in the rotation for half of the years. Rye was not given an off-farm value, but additional costs were added for growing the cover crop after corn.

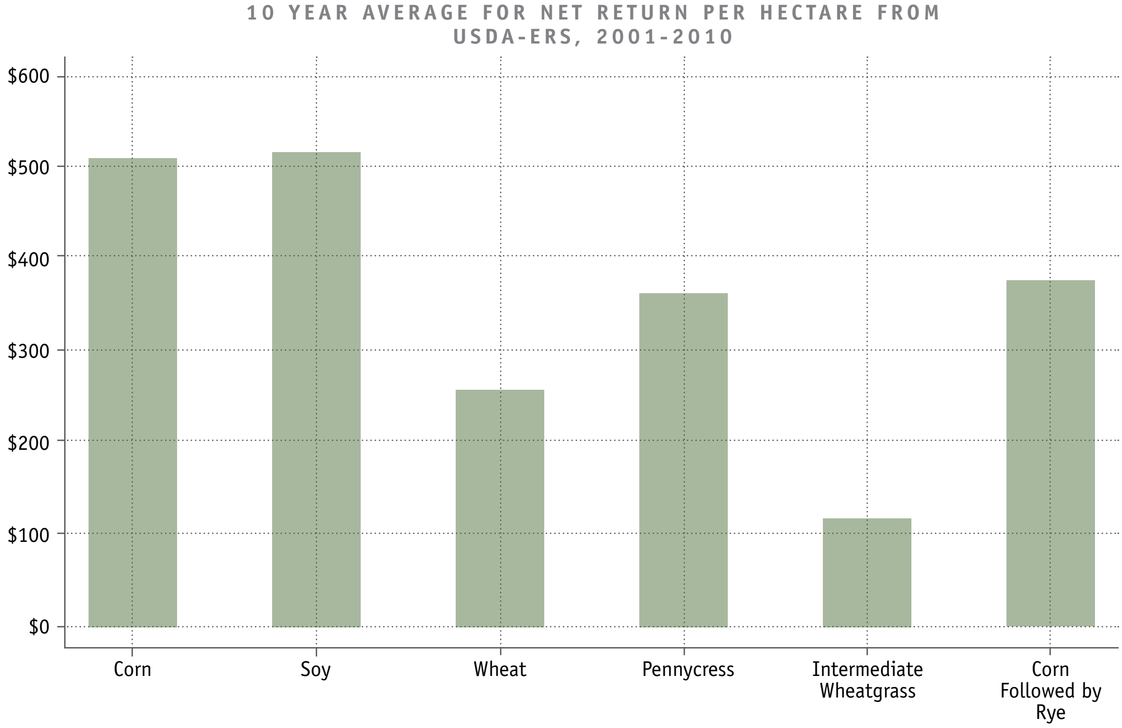
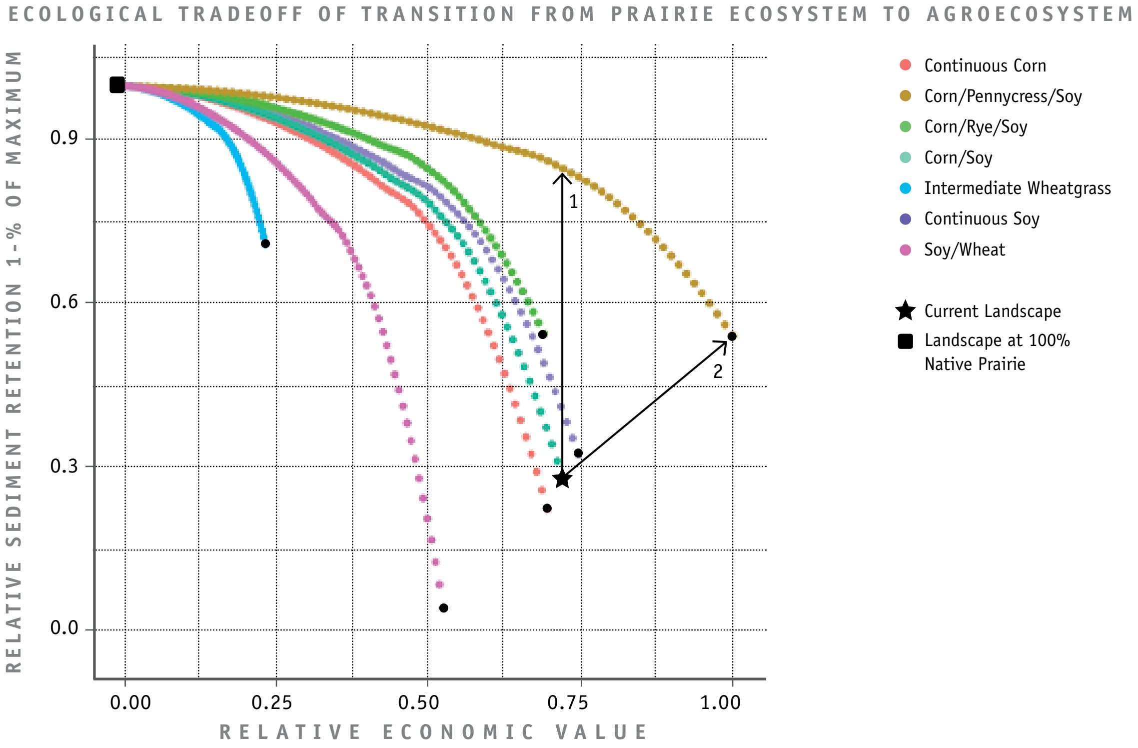


Figure 6 illustrates modelled changes in ecosystem delivery in Watonwan County from 100 percent native prairie to 100 percent managed agro-ecosystem. The relative loss of the ecosystem service of sediment retention was significantly reduced under certain rotations, even though economic output was greatly increased. For example, in the CPS rotation, there is a substantial increase in the delivery of ecosystem services when compared with CS, C, and S. Additionally, while the CRS rotation offered a similar level of sediment retention, the economic output from the CRS system was substantially less than with the CPS rotation. Both SW and IWG underperformed economically compared with corn- and soybean-based rotations; however, the ecological productivity of the IWG was much closer to prairie than any other rotation.

In Figure 6, the star represents an approximation of Watonwan County's current ecosystem service delivery. Black Arrow one shows the sediment retention service gain that could be made without losing any economic output at the county level by switching to a CPS rotation with approximately 15 percent of the landscape remaining in native prairie. Black Arrow two shows the potential economic and ecosystem service gains that would be possible by shifting 100 percent of cropped land from the existing rotation to 100 percent CPS rotation. This situation illustrates one of the major goals of the Forever Green initiative: to develop new material that positively alters both the economic and environmental output of a landscape. Our examination of perennial wheatgrass showed an increase in sediment retention and a reduction in profit compared with other crops. Nevertheless, the difference between intermediate wheatgrass and its closest relative, wheat (Figure 5), is relatively small. Economically, neither performs well against maize or soybean, however.

FIGURE 6. ECOLOGICAL TRADEOFF FOR SEVEN DIFFERENT CROP ROTATIONS AS CROPPED LAND IN WATONWAN COUNTY, MINNESOTA, IS CHANGED FROM 100 PERCENT PRAIRIE TO 100 PERCENT OF EACH OF THE DIFFERENT CROP ROTATIONS

Curves indicate the tradeoff between relative sediment loss and relative economic value of each rotation. The black dot at the end of a curve represents the maximum potential loss, and the star represents the position of the current landscape. The square represents a landscape that is entirely native prairie. Black arrow one shows the sediment retention service gain that could be made without losing any economic output at the county level by switching to a CPS rotation. Black arrow two shows the potential economic and ecosystem service gains that would be possible by shifting 100 percent of cropped land from the existing rotation to 100 percent CPS rotation.



The Forever Green crops that we modelled fit both ends of the spectrum. Intermediate wheatgrass provided excellent environmental benefits, but in its current form did not produce the required profitability. The corn/pennycress/soybean rotation provided an increase in ecosystem services (though not as great as with intermediate wheatgrass) and an increase in profit compared with the current system. This shows that we have existing technologies that can be applied to the landscape, as well as technologies that are on their way to being developed that may have greater environmental benefits.

IMPLICATIONS FOR THE FUTURE

The continued development of new plant material through integrated approaches such as the Forever Green initiative's Reflective Plant-Breeding Paradigm could provide win-win scenarios that deliver the profitability and the ecosystem services that stakeholders desire. In the current plant-breeding paradigm, now largely driven by markets for crops that support profitability, the breadth of ecosystem services desired by society are often overlooked because they lack sufficient profitability. Fortunately, universities and other non-profit organizations can take long-term views and greater risks, and produce outcomes from cropping systems once thought unimaginable. Perennial grains show promise, but at current levels of yield, their adoption by farmers is highly unlikely. While intermediate wheatgrass remains under development, other continuous cover crops such as pennycress appear to be nearly ready for landscape deployment. In the short term, increasing continuous landscape cover though the use of winter annual covers offers a promising avenue to deliver ecological and economic services. Eventually, as these cropping systems and associated supply and value chains (Jordan *et al.* 2013) are made less risky, growers and supply-chain firms may see these "alternative" crops as reasonable for investment. Given the preliminary results of the Watonwan County case study and other research being done at the University of Minnesota, the time of "reasonable for investment" may be close at hand.

REFERENCES

Barnes, R., Lehman, C., Kantar., M.B., DeHaan, L.R. & Wyse, D.L. 2013. P*erennial possibilities: a theory for yield differences between annual and perennial grains*. Presentation: at the 98th annual Ecology Society of America Meeting, Minneapolis, MN, USA.

Barnhart, S., Duffy, M. & Owen, R. February 2012. Estimated costs of pasture and hay production. *Agriculture Decision Maker.* 1-8.

Baulcombe, D., Crute, I., Davies, B., Dunwell, J., Gale, M., Jones, J., Pretty, J., Sutherland, W. & Toulmin, C. 2009. *Reaping the benefits: science and the sustainable intensification of global agriculture*. London: The Royal Society.

Bell, L., Byrne, F., Ewing, M.A. & Wade, L.J. 2008. A preliminary whole-farm economic analysis of perennial wheat in an Australian dryland farming system. *Agricultural Systems.* 96: 166-174.

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W.E, Siemann, E. & Prati, D. 2005. Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia*. 144: 1-11.

Collins, H.P., Smith, J.L., Fransen, S., Alva, A.K., Kruger, C.E. & Granatstein, D.M. 2010. Carbon sequestration under irrigated switchgrass (*Panicum virgatum* L.) production. *Soil Science Society of America Journal.* 74: 2049–2058.

Cox, T.S., Bender, M., Picone, C., Van Tassel, D.L., Holland, J.B., Brummer, E.C., Zoeller, B.E., Paterson, A.H. & Jackson, W. 2002. Breeding perennial grain crops. *Critical Reviews in Plant Sciences.* 21: 59-91.

Creamer, N.G., Bennett, M.A., Stinner, B.R., Cardina, J. & Regnier, E.R. 1996. Mechanisms of weed suppression in cover crop-based production systems. *Hortscience.* 31: 410-413.

Dana, G.V. & Nelson, K.C. 2012. Social learning through environmental risk analysis of biodiversity and GM maize in South Africa. *Environmental Policy and Governance.* 22: 238- 252.

DeHaan, L.R., Van Tassel, D.L. & Cox, T.S. 2005. Perennial grain crops: A synthesis of ecology and plant breeding. *Renewable Agriculture and Food Systems.* 20: 5-14.

Desmet, P. & Grovers, G. 1996. A GIS procedure for automatically calculating the USLE LS factor on topographically complex landscape units. *Journal of Soil and Water Conservation*. 51: 427–433.

Dowle, M., Short, T. & Lianoglou, S. 2013. *Data table: Extension of data frame for fast indexing, fast ordered joins, fast assignment, fast grouping and list columns*. R package version 1.8.8. CRAN.R-project.org/package=data.table

ESRI. 2011. *ArcGIS Desktop: Release 10*. Redlands, CA: Environmental Systems Research Institute.

Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Mueller, N.D., O'Connell, C., Ray, D.K., West, P.C., Balzer, C., Bennett, E.M., Carpenter, S.R., Hill, J., Monfreda, C., Polasky, S., Rockstrom, J., Sheehan, J., Siebert, S., Tilman, D. & Zaks, D.P.M. 2011. Solutions for a cultivated planet. *Nature.* 478: 337–342.

Fry, J., Xian, G., Jin, S., Dewitz, J., Homer, C., Yang, L., Barnes, C., Herold, N., & Wickham, J. 2011. Completion of the 2006 national land cover database for the conterminous United States, *Photogrammetric Engineering & Remote Sensing.* 77: 858-864.

Galzki, J., Birr, A.S. & Mulla, D.J. 2011. Identifying critical agricultural areas with three-meter LiDAR elevation data for precision conservation. *Journal of Soil and Water Conservation.* 66: 423-430.

Gardiner, M.M., Landis, D.A, Gratton, C., DiFonzo, C.D., O'Neal, M., Chacon, J.M., Wayo, M.T., Schmidt, N.P., Mueller, E.E. & Heimpel, G.E. 2009. Landscape diversity enhances biological control of an introduced crop pest in the north-central USA. *Ecological Applications.* 19: 143–154.

Garnett, T., Appleby, M.C., Balmford, A., Bateman, I.J., Benton, T.G., Bloomer, P., Burlingame, B., Dawkins, M., Dolan, L., Fraser, D., Herrero, M., Hoffmann, I., Smith, P., Thornton, P.K., Toulmin, C., Vermeulen, S.J. & Godfray, H.C.J. 2013. Sustainable intensification in agriculture: premises and policies. *Science.* 341: 33-34. DOI:10.1126/science.1234485

Garrity, D.P., Akinnifesi, F.K., Ajayi, O.C., Weldesemayat, S.G., Mowo, J.G., Kalinganire, A., Larwanou, M. & Bayala, J. 2010. Evergreen agriculture: a robust approach to sustainable food security in Africa. *Food Security*. 2: 197-214.

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J. Robinson, S., Sandy, M., Thomas, S.M. & Toulmin, C. 2010. Food security: the challenge of feeding 9 billion people. *Science*. 327: 812-818.

Harlan, J.R. 1992. *Crops and man*. Madison, WI: American Society of Agronomy.

Hogg, B.N., Nelson, E.H., Mills, N.J. & Daane, K.M. 2011. Floral resources enhance aphid suppression by a hoverfly. *Entomologia Experimentalis et Applicata*. 141: 138–144.

Johnson, K.A., Dana, G., Jordan, N.R., Draeger, K.J., Kapuscinski, A., Olabisi, L.K.S. & Reich, P.B. 2012. Using participatory scenarios to stimulate social learning for collaborative sustainable development. *Ecology and Society*. 17: 9.

Jones, G.A. & Gillett, J.L. 2005. Intercropping with sunflowers to attract beneficial insects in organic agriculture. *Florida Entomologist*. 88: 91–96.

Jones, K.B., Neale, A.C., Nash, M.S., Van Remortel, R.D., Wickham, J.D., Riitters, K.H. & O'Neill, R.V. 2001. Predicting nutrient and sediment loadings to streams from landscape metrics: A multiple watershed study from the United States Mid-Atlantic region. *Landscape Ecology*. 16: 301-312.

Jordan, N., Boody, G., Broussard, W., Glover, J.D., Keeney, D., McCown, B.H., McIsaac, G., Muller, M., Murray, H., Neal, J., Pansing, C., Turner, R.E., Warner, K. & Wyse, D. 2007. Sustainable development of the agricultural bio-economy. *Science*. 316(5831): 1570-1571. DOI:10.1126/science.1141700

Jordan, N., Schively-Slotterback, C., Cadieux, K.V., Mulla, D., Schmidt-Olabisi, L., Pitt, D. & Kim, J.O. 2011. TMDL implementation in agricultural landscapes: A communicative and systemic approach. *Environmental Management*. 44:1-12. Published online: March 2011. DOI 10.1007/s00267-011-9647-y

Jordan, N., Williams, C. L., Schulte Moore, D., Pitt, C., Schively-Slotterback, R., Jackson, D., Landis, D., Mulla, D., Becker, M., Rickenbach, B., Dale, C., Helmers, & Bringi, B. 2013. Landlabs: A new approach to creating agricultural enterprises that meet the triple bottom line. *Journal of Higher Education Outreach and Engagement*. 17:176-200.

Kantar, M.B., Betts, K., Michno, J.M., Luby, J.J., Morrell, P.L., Hulke, B.S., Stupar, R.M., Wyse, D.L. 2014. Evaluating an interspecific *Helianthus annuus x Helianthus tuberosus* population for use in a perennial sunflower breeding program. *Field Crops Research*. 155:254–264.

Kaspar, T.C., Jaynes, D.B., Parkin, T.B., Moorman, T.B. & Singer, J.W. 2012. Effectiveness of oat and rye cover crops in reducing nitrate losses in drainage water. *Agricultural Water Management*. 110: 25–33.

Kort, J., Collins, M. & Ditsch, D. 1998. A review of soil erosion potential associated with biomass crops. *Biomass and Bioenergy*. 14: 351-359.

Leavitt, M.J., Sheaffer, C.C., Wyse, D.L. & Allan, D.L. 2011. Rolled winter rye and hairy vetch cover crops lower weed density but reduce vegetable yields in no-tillage organic production. *HortScience*. 46: 387-395.

Letourneau, D.K., Armbrecht, I., Rivera, B.S., Lerma, J.M., Carmona, E.J., Daza, M.C., Escobar, S., Galindo, V., Gutiérrez, C., López, S.D., Mejía, J.L., Rangel, A.M.A., Rangel, J.H., Rivera, L., Saavedra, C.A., Torres, A.M. & Trujillo, A.R. 2011. Does plant diversity benefit agroecosystems? A synthetic review. *Ecological Applications*. 21: 9–21.

Lundgren, J. 2009. Nutritional aspects of non-prey foods in the life histories of predaceous Coccinellidae. *Biological Control*. 51: 294–305.

Luo, Z., Wang, E. & Sun, O.J. 2010. Canno-tillagestimulatecarbonsequestrationin agricultural soils?Ameta-analysisofpairedexperiments. *Agricultural Ecosystem Environment*. 139: 224–231.

Minnesota D.N.R. 2010. *LiDAR Elevation Data for Minnesota*. In LiDAR Elevation Data for Minnesota. Retrieved 15 July 2013. (Available at www.mngeo.state.mn.us/chouse/elevation/lidar.html).

Phippen, W.B. & Phippen, M.E. 2012. Soybean seed yield and quality as a response to field pennycress residue. *Crop Science*. 52: 2767-2773.

Power, A.G. 2010. Ecosystem services and agriculture: Tradeoffs and synergies. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 365: 2959–2971.

Powlson, D.S., Whitmore, A.P. & Goulding, K.W.T. 2011. Soil carbon sequestration to mitigate climate change: A critical re-examination to identify the true and the false. *European Journal of Soil Science*. 62: 42-55.

Mishra, U., Ussiri, D.A.N. & Lal, R. 2010. Tillage effects on soil organic carbon storage and dynamics in Corn Belt of Ohio USA. *Soil & Tillage Research*. 107: 88-96

Mitasova, H., Hofierka, J., Zlocha, M. & Iverson, L. 1996. Modelling topographic potential for erosion and deposition using GIS. *International Journal of GIS*. 10: 629-641.

Teasdale, J.R., Coffman, C.B. & Mangum, R.W. 2007. Potential long-term benefits of no-tillage and organic cropping systems for grain production and soil improvement. *Agronomy Journal*. 99: 1297-1305.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R. & Polasky, S. 2002. Agricultural sustainability and intensive production practices. *Nature*. 418: 671-7.

Tilman, D., Balzer, C., Hill, J. & Befort, B.L. 2011. Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences of the United States of America*. 108: 20260-20264.

R Core Team. 2013. R: *A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. (Available at www.R-project.org/).

Rabia, A.H. 2012. Mapping soil erosion risk using RUSLE, GIS and remote sensing techniques. The 4th International Congress of ECSSS, *EUROSOIL*. Bari, Italy.

Renard, K.G., Foster, G.R., Weesies, G.A. & Porter, J.P. 1991. RUSLE: Revised universal soil loss equation. *Journal of Soil and Water Conservation*. 46: 30-33.

Runge, F.C., Senauer, B., Pardey, P.G. & Rosegrant, M.W. 2003. *Ending Hunger in Our Lifetime: Food Security and Globalization*. Baltimore: John Hopkins University Press.

Scheinost, P.L., Lammer, D.L., Cai, X., Murray, T.D. & Jones, S.S. 2001. Perennial wheat: the development of a sustainable cropping system for the US Pacific Northwest. *American Journal of Alternative Agriculture*. 16: 147-151.

Soil Survey Staff. 2013. *The Gridded Soil Survey Geographic (gSSURGO) Database for Minnesota*. U.S. Department of Agriculture, Natural Resources Conservation Service. (Available at http://datagateway.nrcs.usda.gov/). July 2013 (FY2013 official release).

Strock, J.S., Porter, P.M. & Russelle, M.P. 2004. Cover cropping to reduce nitrate loss through subsurface drainage in the northern U.S. Corn Belt. *Journal of Environmental Quality*. 33: 1010-1016.

USDA ERS. 2013. *Commodity Costs and Returns*. In Economic Research Service: United States Department of Agriculture. Retrieved 24 July 2013. (Available at www.ers.usda.gov/data-products/commodity-costs-and-returns.aspx#.Uf_CxOL2Si1).

Vaché, K.B., Eilers, J.M. & Santelmann, M.V. 2002. Water quality modeling of alternative agricultural scenarios in the U.S. corn belt. *Journal of the American Water Resources Association*. 38(3): 773–787

Vaughan, D.A., Balazs, E. & Heslop-Harrison, J.S. 2007. From crop domestication to superdomestication. *Annals of Botany*. 100(5): 893-901.

Wallander, S. 2013. *While Crop Rotations Are Common, Cover Crops Remain Rare*. www.ers.usda.gov. Retrieved 7 July 2013. (Available at www.ers.usda.gov/amber-waves/2013-march/while-crop-rotations-are-common,-cover-crops-remain-rare.aspx#.UeAmWW2jd8F).

Warner, K.D. 2007. *Agroecology in action: Extending Alternative Agriculture through Social Networks*. The MIT Press.

Weeden, N.F. 2007. Genetic changes accompanying the domestication of *Pisum sativum*: is there a common genetic basis to the 'domestication syndrome' for legumes? *Annals of Botany*. 100: 1017–1025.

Wickham, H. 2009. *ggplot2: elegant graphics for data analysis*. Springer, New York.

Wickham, H. 2011. The split-apply-combine strategy for data analysis. *Journal of Statistical Software*. 40: 1-29. (Available at www.jstatsoft.org/v40/i01/).

Wischmeier, W.H. & Smith, D.D. 1978. *Predicting rainfall erosion losses - guide to conservation planning*. U.S. Department of Agriculture, Agricultural Handbook No. 537.

Zilberman, D., Hochman, G., Rajagopal, D., Sexton, S. & Timilsina, G. 2013. The impact of biofuels on commodity food prices: assessment of findings. *American Journal of Agricultural Economics*. 95: 275-281.

APPENDIX 1. **METHODOLOGY FOR WATONWON COUNTY, MINNESOTA, CASE STUDY.**

We chose Watonwan County in southern Minnesota to explore our cropping system scenarios because it represents highly productive land similar to that across much of the United States Corn Belt. The data for RUSLE was easily accessed from public sources of information. RUSLE is an empirically derived model that estimates rill and inter-rill erosion in tonnes/ha*yr (A) as a function of flow length in metres (L), slope in dimensionless units (S), rainfall and runoff erosivity index in MJ*mm/ha*yr (R), inherent soil erodibility in dimensionless units (K), cover type in dimensionless units (C), and supporting conservation practices in dimensionless units (S) (Renard *et al.* 1991; Desmet and Grovers, 1996) so that:

$$A = L*S*R*K*C*S.$$

We downloaded the baseline digital elevation model of Watonwan County from the Minnesota Department of Natural Resource's data warehouse in order to calculate the slope length and steepness (LS factor). The DEM was derived from Light Detection and Ranging (LiDAR) data captured in the spring of 2010 and downloaded orthorectified at a 1 metre spatial resolution in Nicotinamide adenine dinucleotide (NAD) 83 Universal Transverse Mercator (UTM) 15 coordinate system (retrieved July 2013). For further details on the creation of the DEM, refer to the online metadata (Minnesota DNR, 2010). The K factor was taken from the gridded Soil Survey Geographic (gSSURGO) database downloaded from the Natural Resources Conservation Services (NRCS) Data Gateway *(***Soil Survey Staff, 2013***)*. Data to calculate the R factor was found in the Agricultural Handbook (AH) 537 for Watonwan County (Wischmeier and Smith, 1978). The C factor was derived using the method found in AH 537 (Wischmeier and Smith, 1978), and because no supporting practices are being assessed for this study, the S factor was determined to be 1. All data was cropped to the area of interest and reprojected in the NAD 83, UTM zone 15 coordinate system in the Esri Geographic Information System software (ArcGIS) 10.0 (ESRI, 2011). The digital elevation model was resampled by interpolation to a 10 m^2 spatial resolution to correspond to the gSSURGO database. The LS factor was calculated entirely in ArcGIS 10.0 (ESRI, 2011) by first calculating the slope from the DEM using the slope tool. Flow direction was calculated from the DEM using the flow direction tool, and from the flow direction raster, flow accumulation was calculated. Following the recommendations established in Desmet and Grovers (1996) and Mitasova *et al.* (1996), flow length was replaced with flow accumulation, and then the LS factor was calculated using the equation:

$$LS = (\frac{A}{a_o})^m(\frac{S}{s_o})^n$$

where A is flow accumulation, a_0 is 22.13 metres based on the length of original test plots, S is slope, s_0 is 0.09 based on the slope of the original tests plots, and m and n are 1.4 and 0.5 – constants determined by empirical testing or the literature (Rabia, 2012). The equation was calculated using the raster calculator tool resulting in an LS raster. The K factor was then isolated from the gSSURGO database, and multiplied by the LS raster resulting in an LSK raster.

The C factor for each of the seven rotations and native prairie was calculated by the method established in AH 537 (Wischmeier and Smith, 1978) for a ten-year period. Utilizing the data table (Dowle *et al.* 2013), plyr (Wickham, 2011), and stats (R Development Core Team, 2012) packages in R version 3.0.1 (R Development Core Team, 2013), the C factors were each multiplied by the LSKR factors and then divided by 1 000 to give sediment movement (A) under the different cropping systems for each 100 m^2 grid cell. A was then put on a relative to maximum scale across all rotations.

In order to isolate the cropped land, the NLCD data layer was reclassified using the raster reclassification tool where classes 81 (Hay/Pasture) and 82 (Row Crops) were one and all else was 0. Using the raster algebra tool, the reclassified NLCD layer was multiplied by the LSK raster and the R factor from AH 537 to result in a cropland LSKR raster. This raster was then resampled to a 100 m spatial resolution and exported as a CSV file with a key field, the LSKR calculation, and the Crop Productivity Index (CPI) for each grid cell derived from the gSSURGO database.

To calculate the net economic return for each cropping system from 2001 to 2010, we utilized crop production economic data containing average yield, production costs, gross return per acre (converted to gross return per hectare), net return per acre (converted to gross return per hectare), and price per bushel from the USDA-ERS (retrieved July 2013). We calculated the net profit per kilogram of yield. The value of pennycress was calculated as 50 percent of the value of soybean, and intermediate wheatgrass as 85 percent the value of wheat. Input costs were modified to represent pennycress being in the rotation five of ten years, and intermediate wheatgrass having seeding costs only twice in the ten year period. We assumed intermediate wheatgrass would develop with the first year for establishment resulting in full input costs without any grain produced. Subsequent years were assumed to have reduced input costs and full yield until year six when it would need to be reseeded. Rye was not given an off farm value, but additional costs were added for growing the cover crop after corn.

Yield for each crop within each grid cell was determined by creating three random normal distributions – a high, average, and low (Figure 2) - built from the USDA-ERS 2001 to 2010 data and empirical data collected from 2006 to 2012 in Minnesota for pennycress and intermediate wheatgrass as a part of the Forever Green program, additional data for biomass value was gathered from Barnhart *et al.* (2012). The average distribution for each crop was based off of the mean and standard deviation of yield for the respective crop. The high and low distribution means were determined as the mean of the average plus or minus 1.5 times the standard deviation. The standard deviation from the average distribution was used in the high and low. Using the CPI for each grid cell in Watonwan County, yield was chosen at random from the appropriate distribution for each crop. This process resulted in a spatially informed yield for each grid cell.

Crop yield for each grid cell was then multiplied by the average value of the crop over a ten year period, and then adjusted to represent the respective value in rotation with other crops. The valuation resulted in a net rotation return per grid cell. Net value of a rotation was chosen because it captures what landowners would gain for themselves after the costs of production were met, and gives a sense of what type of livelihood can be made from the landscape under a given cropping system.

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ARE PERENNIAL CROPS MORE ADAPTED TO MAINTAIN LONG-TERM RELATIONSHIPS WITH SOILS AND, THEREFORE, TO SUSTAINABLE PRODUCTION SYSTEMS, SOIL RESTORATION AND CONSERVATION?

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ABSTRACT

Developing perennial crops involves many challenges, such as combining perenniality with high yield. However, attention also needs to be given to the sensitivity of perennial crops to tolerance for or resistance against pests and pathogens. Here, I discuss why it is important to consider soil-borne enemies and propose three avenues for further research.

Keywords: learning from nature, succession, plant traits, soil food webs, pathogens, ecosystem processes

Most major food and feed crops in the world have been derived from annual early successional plant species (Diamond, 1997). Traits that characterize early successional plant species are ephemeralism, preference for disturbed sites, low nutrient use efficiency, and pathogen sensitivity, however, such trait combinations in crops require crop rotation, land tillage, fertilization, and biocide use to control belowground and aboveground pests and pathogens. These requirements are a major constraint for sustainable agriculture, as they result in production of greenhouse gasses, loss of organic matter, nutrient leaching to ground- and surface water, and pollution of the environment with toxic biocides. Developing perennial crops could be a solution for circumventing intensive soil disturbance. However, early successional perennials also have adverse trait combinations that require intensive management practices. The question is what may be learned from nature when aiming at producing sustainable perennial crop production systems. I will discuss some research highlights on secondary succession following land abandonment in order to elucidate how soil food webs and soil ecosystem processes may respond to both changes in management and plant trait characteristics of early, mid, and late successional annual and perennial plant species.

Reducing land tillage and fertilization results in a development of the soil biodiversity and soil food web composition, which affects the mineralization and cycling of nutrients in ecosystems (De Vries *et al.* 2013). Such ecosystems could also be more resistant to extreme events, such as drought stress during the growing season (De Vries *et al.* 2012). Changes in soil food web composition and functioning are to some extent related to the presence of plant species with specific traits (Bezemer *et al.* 2010), whereas in part they are due to successional developments that are the result of reduced intensity of land use practices (Holtkamp *et al.* 2011). Insights from (semi-) natural ecosystems may stimulate thinking about how perennial crops could be developed in such a way that they will further enhance the sustainability of agriculture.

In a series of studies on the contribution of soil biota to vegetation development on abandoned ex-arable land, it was shown that early successional plant species had negative feedback interactions with the soil biota, both with soil fauna (De Deyn *et al.* 2003) and soil microbes (Kardol *et al.* 2006). Negative plant-soil feedback means that plants stimulate pathogenic components in the soil community more than symbiotic or mutualistic components, such as arbuscular mycorrhizal fungi (Bever *et al.* 1997). These negative feedback effects were not only evident in annual plant species, but also in (short-lived) perennials (Van de Voorde *et al.* 2011). Some plant species had positive feedback with the soil community, but those effects were more confined to later successional, slow growing plant species (Kardol *et al.* 2006). These species appeared to be promoted by the soil biota that were developing in their rhizosphere. However, this trait turned out to be combined with slow growth, which will be less desirable for plant species that are targeted for primary production.

Whereas these results are based on studies on non-cultivated (wild) plant species and mostly limited to temperate habitats, an increasing amount of studies is showing that negative plant-soil feedbacks occur in many early successional plant communities, independent of climate and soil type (van der Putten *et al.* 2013). Therefore, if annual crops are being developed into perennial crops, the advantage of perenniality, which will undoubtedly benefit the sustainability of soils, soil biodiversity, and counteract soil erosion due to reduced soil tillage, might be counteracted by the fact that crop ageing may go hand in hand with yield declines due to increasing exposure to soil-borne enemies, such as pathogens, root-feeding nematodes and herbivorous insect larvae.

There are several possibilities for counteracting these unwanted side effects of perenniality in crops, which may be accounted for in crop developing programmes. Thus far, there is little attention for these aspects and the question is how they may be accounted for. Here, I will provide three suggestions, which may need to be explored in subsequent studies. First, perennial crop varieties may vary in their susceptibility for negative plant-soil feedback development. Testing this would require screening of potential crop varieties in soils while allowing negative plant-soil feedback to occur. Recording effects of ongoing growth on temporal yield development and repetitive sowing in these soils may provide insight in the development of growth reducing soil biota. Second, the rhizosphere microbiome (Mendes *et al.* 2011) may be examined in order to test effects of perennial crop varieties on the development of a microbial community that may be antagonistic to major soil-borne pathogens and herbivores. Third, perennial crops may need to be grown in rotation, just as is being done with annual crops, in order to reduce the potential of soil-borne enemies between subsequent growth cycles. The main difference with current agriculture would be that crop rotations take many years, as each crop will be grown for several years in a row.

In conclusion, I wish to emphasize that the development of perennial crops may require prevention of negative side effects, such as the development of soil-borne pathogens. Perennial crops still have characteristics of early successional plant species, which make them sensitive to soil-borne enemies and, possibly, also aboveground pests and pathogens. Perennial crop development programmes, therefore, need to account for these unwanted side effects and I have proposed three avenues, but there will be clearly more possibilities to explore. The main point is that perennial crop development not only has to pass the hurdle of developing perennial varieties, but also of testing these varieties for resistance against, or tolerance of belowground and aboveground pests and pathogens. When accounting for these additional requirements, screening programmes may avoid future problems with e.g. yield declines in later years of perennial crop growth cycles.

REFERENCES

Bever, J.D., Westover, K.M. & Antonovics, J. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology*. 85: 561-573.

Diamond, J.M. 1997. *Guns germs and steel*. W.W. Norton & Company, New York.

De Vries, F.T., Thébault, E., Liiri, M., Birkhofer, K., Tsiafouli, M.A., Bjørnlund, L., Bracht Jørgensen, H., Brady, M.V., Christensen, S., de Ruiter, P.C., d'Hertefeldt, T., Frouzk, J., Hedlund, K., Hemerik, L., Hol, W.H.G., Hotes, S., Mortimer, S.R., Setälä, H., Sgardelis, S.P., Uteseny, K., van der Putten, W.H., Wolters, V., & Bardgett, R.D. 2013. Soil food web properties explain ecosystem services across European land use systems. *Proceedings of the National Academy of Sciences*. 110: 14296-14301.

De Vries, F., Liiri, M., Bjørnlund, L., Bowker, M., Christensen, S., Setälä, H. & Bardgett, R. 2012. Land use alters the resistance and resilience of soil food webs to drought. *Nature Climate Change*. 2: 276-280.

Bezemer, T.M., Fountain, M.T., Barea, J.M., Christensen, S., Dekker, S.C., Duyts, H., Van Hal, R., Harvey, J.A., Hedlund, K., Maraun, M., Mikola, J., Mladenov, A.G., Robin, C., De Ruiter, P.C., Scheu, H., Setälä, S., Šmilauer, P., & Van der Putten, W.H. 2010. Divergent composition but similar function of soil food webs of individual plants: plant species and community effects. *Ecology*. 91: 3027-3036.

Holtkamp, R., van der Wal, A., Kardol, P., van der Putten, W.H., de Ruiter, P.C. & Dekker, S.C. 2011. Modelling C and N mineralisation in soil food webs during secondary succession on ex-arable land. *Soil Biology & Biochemistry*. 43: 251-260.

De Deyn, G.B., Raaijmakers, C.E., Zoomer, H.R., Berg, M.P., De Ruiter, P.C., Verhoef, H.A., Bezemer, T.M. & Van der Putten, W.H. 2003. Soil invertebrate fauna enhances grassland succession and diversity. *Nature*. 422: 711-713.

Kardol, P., Bezemer, T.M. & van der Putten, W.H. 2006. Temporal variation in plant-soil feedback controls succession. *Ecology Letters*. 9: 1080-1088.

Mendes, R., Kruijt, M., De Bruin, I., Dekkers, E., Van der Voort, M., Schneider, J.H.M., Piceno, Y.M., DeSantis, T.Z., Andersen, G.L., Bakker, P.A.H.M., & Raaijmakers, J.M. 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science*. 332: 1097-1100.

Van de Voorde, T.F.J., Van der Putten, W.H., & Bezemer, T.M. 2011. Intra- and interspecific plant-soil interactions, soil legacies and priority effects during old-field succession. *Journal of Ecology*. 99: 945-953.

Van der Putten, W.H., Bardgett, R.D., Bever, J.D., Bezemer, T.M., Casper, B.B., Fukami, T., Kardol, P., Klironomos, J.N., Kulmatiski, A., Schweitzer, J.A., Suding, K.N., Van de Voorde, T.F.J., & Wardle, D.A. 2013. Plant-soil feedback: the past, the present and future challenges. *Journal of Ecology*. 101: 265-276.

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PERENNIAL GRAIN SYSTEMS: A SUSTAINABLE RESPONSE TO FUTURE FOOD SECURITY CHALLENGES

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ABSTRACT

Although conventional agricultural systems have provided growing supplies of food and other products, they have also been major contributors to global greenhouse gases, biodiversity loss, natural resource degradation, and public health problems. Concerns about the long-term sustainability of agriculture, especially in light of a growing population, have promoted interest in new transformative approaches to agriculture. Transformative approaches meet FAO's multiple goals of sustainable intensification: increasing crop production per unit area and enhancing environmental, economic, and social sustainability. Perennial grain systems are examples of such innovative systems but perennial grains, such as wheat and maize, will not be commercially operational for at least 15 to 20 years. For any perennial grain to be commercially available by 2030, more resources are needed to (i) accelerate plant breeding programmes with more personnel, land, and technological capacity; (ii) expand agro-ecological research on improved perennial germplasm; (iii) coordinate global activities through germplasm and scientist exchanges and conferences; (iv) identify global priority croplands; and (v) develop training programmes for scientists and students in the breeding, ecology, and management of perennial crops. In addition, farmer involvement, public-private collaborations, and significant changes in markets and policies will be necessary. Large investments have been committed to developing technologies for biofuel conversion of perennial crops because of their ecological advantages compared to annual sources, despite their potential to displace food crops. With similar commitments for developing food-producing perennial grains, commercially viable perennial grain crops could be available by 2030.

Keywords: agricultural research investment, ecosystem services, perennial grains, sustainability indicators, sustainable agriculture, transformative farming systems

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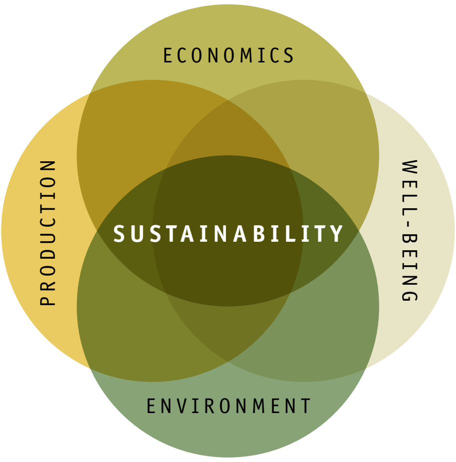
THE MULTIPLE GOALS OF SUSTAINABLE AGRICULTURE

With increasing population pressure and finite resources, is it possible to meet both global food security needs and sustainability needs? According to Foley *et al*. (2011), tremendous progress could be made by (i) halting agricultural expansion, (ii) closing “yield gaps” on underperforming lands, (iii) increasing agricultural resource efficiency, (iv) shifting diets, and (v) reducing waste. Together these strategies could double food production while greatly reducing the environmental impacts of agriculture. Perennial grains could directly address (ii) and (iii).

To do so requires transformative farming systems to address global food security challenges. Why transformative? Because so many serious problems in agriculture exist as a result of not addressing multiple sustainability goals. According to a National Research Council report (2010) from the U.S. National Academy of Sciences, the multiple goals of sustainable agriculture are to (1) provide abundant, affordable food, feed, fibre and fuel; (ii) enhance the natural-resource base and environment; (iii) make farming financially viable, and (iv) contribute to the well-being of farmers, farm workers and farm communities. The National Research Council definition has similarities to that of FAO’s “sustainable intensification”, which is defined as increasing crop production per unit area and improving environmental, economic and social sustainability via management of biodiversity and ecosystem services (FAO, 2008). Sustainability is thus the intersection among economics, well-being, production, and environment (Figure 1).

FIGURE 1. THE FOUR COMPONENTS OF AGRICULTURAL SUSTAINABILITY



INCREMENTAL AND TRANSFORMATIVE APPROACHES TO SUSTAINABLE AGRICULTURE

The National Research Council report (2010) criticised mainstream, conventional farming for not addressing multiple sustainability goals. It identified numerous examples of innovative farming systems and practices that contribute to multiple sustainability goals, but noted they are not widespread. In order to improve the sustainability of U.S. agriculture, the National Research Council Report proposed both incremental and transformative approaches.

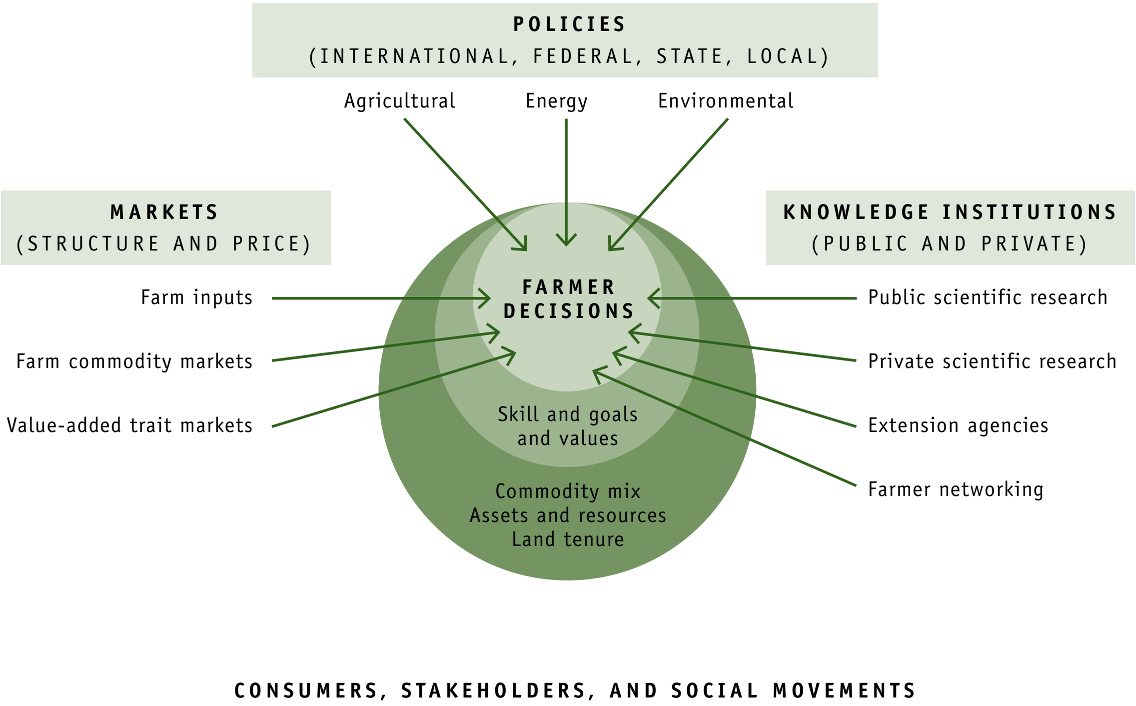
Incremental approaches are practices and technologies that address specific production or environmental concerns associated with mainstream conventional farming systems. Examples include two-year rotations, precision agriculture, classically bred or genetically engineered crops, and reduced or zero tillage. Incremental approaches offer improvements and should continue, but individually, are inadequate to address multiple sustainability concerns.

Conversely, transformative agricultural systems integrate production, environmental, and socioeconomic objectives and reflect greater awareness of ecosystem services on large, mid-size, and small farms. Examples include conservation agriculture, organic farming, mixed crop/livestock farming, integrated (hybrid) systems, agroforestry, and perennial grains.

COEXISTENCE OF DIFFERENT FARMING SYSTEMS

The future requires a coexistence of different farming systems that are sustainable. No one farming system will safely feed the planet, but rather a blend of farming systems will be needed. Proper alignment and coexistence of different farming systems at the landscape level will likely play a key role in future food and ecosystem security. The existence of innovative agricultural systems suggests that technical obstacles are not the greatest barrier. Rather, change is hindered by market structures, policy incentives, and uneven development and availability of scientific information that guide farmers' decisions (Reganold *et al*. 2011) (Figure 2).

FIGURE 2. DRIVERS AND CONSTRAINTS AFFECTING FARMERS' DECISIONS



An illustration of farmers embracing this decision-making process and striving for sustainability is Shepherd's Grain, a marketing label and alliance of a group of farmers in the U.S. Pacific Northwest, who use sustainable production practices and market differentiated wheat products together. Shepherd's Grain was founded by Karl Kupers and Fred Fleming, two U.S. direct-seed farmers from the large commercial grain-producing Palouse region in the states of Washington and Idaho. It has drawn growing attention from agrifood researchers and activists as an example of new "value chains" that can help support an "agriculture of the middle." Shepherds' Grain growers tend the soil and harvest wholesome wheat from farms across the Palouse but have to meet certain sustainability criteria, as defined and certified by the Food Alliance in Portland, Oregon. Shepherd's Grain wheat flours are sold in local health food stores throughout the U.S. Pacific Northwest and northern California and purchased by consumers for their quality, localness, and sustainability certification brand.

THE NEED FOR FARMING SYSTEMS RESEARCH

Unfortunately, most federal research grant programmes in the U.S. and globally still primarily support incremental research. For example, the bulk of public and private agricultural science in the U.S. is narrowly focussed on productivity and efficiency, particularly on technologies that fit into existing production systems and lead to private benefits (Reganold *et al*. 2011). We need to reallocate public funds to support transformative farming systems and systems research that measures multiple sustainability indicators at field, farm, and landscape scales.

Specifically concerning perennial grain systems, we need more studies as only relatively few have been conducted on perennial grains (e.g. Bell *et al*. 2008; Snapp *et al*. 2010; Hayes *et al*. 2012; Jaikumar *et al*. 2012). Moreover, we need farming system comparison studies, with replicates on a commercial farm or experiment station, or with commercial farms as replicates, in which early varieties of perennial grains are grown by themselves, in polycultures with other perennial grains, or in rotation with annual grains.

Such farming system studies require metrics for evaluating and measuring quantifiable components of a farming system. Since we would like a farming system to achieve multiple sustainability or ecosystem service goals, we can measure sustainability indicators or ecosystem services. Measuring a suite of sustainability indicators yields valuable results of a farming system's performance and health. Examples of indicators that can be used for measuring a farm's sustainability are listed in Figure 3. Of the four legs of sustainability – economics, well-being (social), production, and environment – the social sustainability indicators have been the least evaluated in comparison studies (Reganold, 2013).

FIGURE 3. EXAMPLES OF SUSTAINABILITY INDICATORS

SUSTAINABILITY INDICATORS

CROP/ANIMAL YIELD AND QUALITY
yield; plant nutrients; protein content; storage potential; vitamins; weed populations; insect pressure; disease

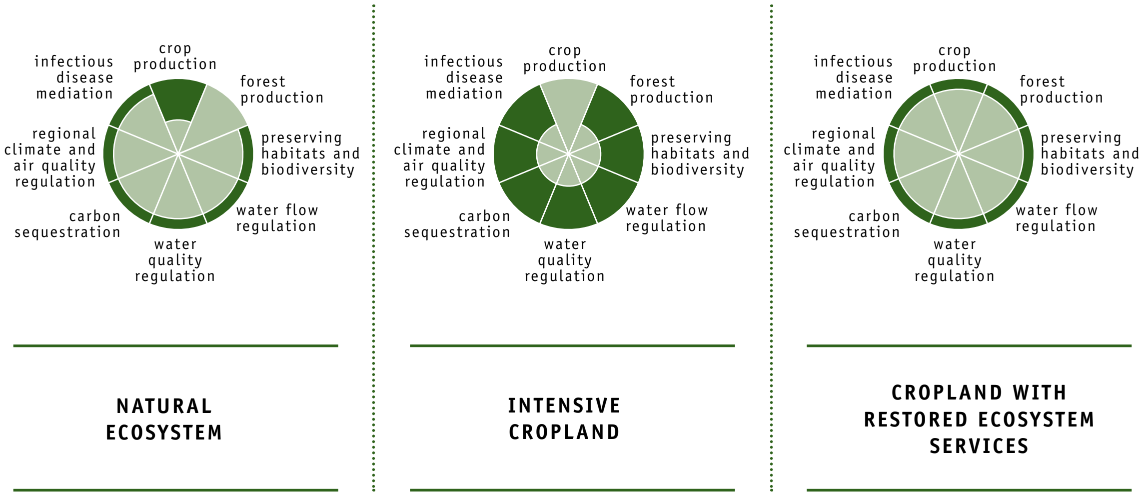
FINANCIAL PERFORMANCE
benefit/cost ratios; net returns; breakeven points; externalities

ENVIRONMENTAL QUALITY
soil health; pesticide and fertilizer impacts; soil erosion; surface and ground water pollution; energy efficiency

SOCIAL JUSTICE
worker wages, benefits, and safety; community well-being

In ecosystem studies, scientists have used ecosystem services as metrics. Examples of ecosystems services that can be measured on farms or plots are crop production, preserving habitats and biodiversity, water flow regulation, water quality regulation, carbon sequestration, air quality regulation, and infectious disease mediation. Figure 4 provides a good example by Foley *et al*. (2005), who illustrate ecosystem services under three contrasting land-use regimes: natural ecosystem, intensive cropland, and cropland with restored ecosystem services (Fig. 4).

FIGURE 4. COMPARING ECOSYSTEM SERVICES UNDER THREE LAND-USE REGIMES



Source: Reganold *et al.*, 2011

One could also measure a combination of sustainability indicators and ecosystem services. A good example of this is research by Glover *et al.* (2010), who evaluated sustainability indicators and ecosystem services between conventionally farmed grain fields and organically managed perennial grasslands at a range of spatial and temporal scales. First, they used commercial paired farm fields as replicates to evaluate ecosystem components of conventionally farmed grain fields and adjacent organically managed perennial grasslands. To make more refined determinations at smaller scales, they initiated replicated treatments on one of the farms. They also used watershed replicates in which the commercial farm replicates were embedded to make other larger-scale determinations.

THE CASE FOR PERENNIAL GRAINS

Farmers in this relatively young millennium face compounding pressures to meet the food needs of a growing, more demanding human population while reducing and reversing the extensive land degradation related to agriculture. Humans have more than doubled the yields of major grain crops over the past 60 years, and yet roughly one in seven people suffer from malnutrition (FAO, 2009). As the global population continues to grow, the demand for food, especially meat, also increases. Additionally, production of nonfood goods (e.g. biofuels) increasingly competes with food production for land and much of the land most suitable for annual crops is already in use (Godfray *et al.* 2010). Global food security largely depends on these annual grains—cereals, oilseeds, and legumes—that are planted on almost 70 percent of croplands and supply a similar portion of human calories. Three annual crops alone—maize, rice, and wheat—provide over 60 percent of human calories. Their production, though, often compromises essential ecosystem services, pushing some beyond sustainable boundaries (Cassman and Wood, 2005; Glover *et al.* 2010).

Current annual cereal crop production on large areas of marginal lands, particularly those on steeply sloping croplands, results in further land degradation and is unlikely to be sustainable over the long term (Cassman *et al.* 2003). These areas are much more suitable for perennial crops, such as forages and biofuels. Unfortunately, food security concerns and/or the greater market value of staple grains often pressure farmers to choose to plant annual grain crops instead of perennial crops even on lands poorly suited to their production. For these farmers, there are too few options to simultaneously meet their food, income, and ecosystems security needs. Meanwhile, the health of their farms continues to deteriorate at the very time that increased grain yields are critical. Facing the triple threats of climate change, land degradation, and a growing human population, business-as-usual approaches to transforming agriculture are no longer acceptable.

Perennial versions of the major grain crops, cereals, grain legumes, and oilseeds, would offer farmers more opportunities to meet their food and income needs while protecting their natural resources even on lands poorly suited to annual crop production. This is not an entirely new idea. Pioneering Russian scientists in the 1930s started perennial wheat breeding programmes and were followed by efforts in the United States in the 1960s (Cox *et al*. 2006). The technologies and resources of the time though limited the success of these programmes. The perennial wheat breeding efforts, for example, were abandoned in part because of plant sterility and undesirable agronomic characteristics (Cox *et al*. 2006). More recently, programmes have been initiated in Argentina, Australia, China, India, Nepal, Sweden, and the United States to identify and improve, for use as grain crops, perennial species and hybrid plant populations derived from annual and perennial parents: rice, wheat, maize, sorghum, pigeon peas, and oilseed crops from the sunflower, flax, and mustard families (Glover *et al*. 2010).

While perennial plant breeding programmes may not produce wide-scale impacts in farmers' fields for another 15 to 20 years, there is emerging evidence that novel perennial grain-based systems provide unique opportunities for protecting water and soil resources, while addressing the pressing problem of climatic variability. Even on the best croplands, perennial crops typically sequester more carbon, better protect soil and water resources, are more resilient to climatic changes, and are more productive above- and below-ground (Cox *et al*. 2006). Compared to annual crops, perennials have the potential to double sequestered carbon, and some can fix nitrogen. The extensive root systems and vegetative cover of perennial crops are the biological foundation to a 'climate smart' agriculture that captures and utilizes water resources, rehabilitates soil, and sequesters carbon. At the same time, food production must be a priority in the design of farming systems. This ensures immediate returns in the form of food security and economic benefits, in addition to environmental services from well-designed combinations of perennial, semi-perennial, and annual crops. Development of perennial grain crops has been termed the missing ingredient, as staple crops have historically been dominated by annual life forms (van Tassel *et al*. 2010).

RECOMMENDATIONS AND CONCLUSIONS

Large investments have been committed to developing technologies for biofuel conversion of perennial crops, despite their potential to displace food crops. With similar commitments for developing food-producing perennial grains, commercially viable perennial grain crops could be available by 2030. Public policies (e.g. the United States Farm Bill) and private funding are needed to support perennial grain systems. However, different strategies will be necessary to get funding for perennial grain development in specific countries, especially in developing compared to developed countries.

For any perennial grain to be commercially available by 2030, more resources are needed to do the following:

1. Accelerate plant breeding programmes with more personnel, land, and technological capacity;
2. Expand agro-ecological research on improved perennial germplasm; for example, we need perennial grain farming systems research on large plots and commercial-sized farm fields,

which in turn can generate confidence in further research investment. Such systems studies can be comparison studies with annual grain or mixed perennial/annual grain systems;

3. Support farmer involvement and develop public-private collaborations;
4. Coordinate global activities through germplasm and scientist exchanges and conferences;
5. Develop training programmes for scientists and students in the breeding, ecology, and management of perennial crops; and
6. Establish a World Perennial Grain Research Centre where resources can be focussed, priorities identified, and information and germplasm exchanged.

We need to change the discussion from annual versus perennial to complementary blends of the two. In addition, we need to better sell perennial grain systems based on their multiple sustainability benefits for global food security. Along these lines, including externalities and ecosystem services in economic studies would illustrate the financial viability of perennial grain systems. Perennial grains need to be more demand-driven by national governments, research institutes, and farmers and less supply-driven by institutions in developed countries. We need a systematic analysis of the highest potential perennial grain crops in development and the potential regions and global priority croplands where they are needed the most or can grow best.

Finally, we need to better communicate about perennial grains. If we want to reach farmers, producers, consumers, and extension agencies, social media utilities, such as YouTube videos, blogs, Facebook, and webpages, need to be used. Outreach events, such as field days and presentations, are also important. Perennial grain research findings from journals need to be reported in extension and outreach bulletins, articles in popular trade journals, and government technical guides and fact sheets.

REFERENCES

Bell, L.W., Byrne, F., Ewing, M.A. & Wade, L.J. 2008. A preliminary whole-farm economic analysis of perennial wheat in an Australian dryland farming system. *Agricultural Systems*. 96: 166–174.

Cassman, K.G., Dobermann, A., Walters, D.T. & Yang, H. 2003. Meeting cereal demand while protecting natural resources and improving environmental quality. *Annual Review of Environment and Resources*. 28: 315-358.

Cassman, K.G. & Wood, S. 2005. Cultivated Systems. In: Hassan, R., Scholes, R. & Ash, N. (eds.) *Millennium Ecosystem Assessment. Ecosystems and Human Well-being: Current State and Trends*, Volume 1. Island Press, Washington D.C. pp. 745-794.

Cox, T.S., Glover, J.D., Van Tassel, D.L., Cox, C.M. & DeHaan, L.R. 2006. Prospects for developing perennial grain crops. *BioScience*. 56: 649-659.

FAO. 2008. *Investing in Sustainable Agricultural Intensification. The Role of Conservation Agriculture. A Framework for Action*. Food and Agriculture Organization of the United Nations. Rome, Italy.

FAO. 2009. *The State of Food Insecurity in the World 2009. Economic Crises—Impacts and Lessons Learned*. FAO, Rome. (Available at ftp://ftp.fao.org/docrep/fao../012/i0876e/i0876e.pdf).

Foley, J.A. Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Mueller, N.D., O'Connell, C., Ray, D.K., West, P.C., Balzer, C., Bennett, E.M., Carpenter, S.R., Hill, J., Monfreda, C., Polasky, S., Rockström, J., Sheehan, J., & Siebert, S., Tilman, D. & Zaks, D.P.M. 2011. Solutions for a cultivated planet. *Nature*. 478: 337–342.

Glover, J.D., Culman, S.W., DuPont, S.T., Broussard, W., Young, L., Mangan, M.E., Mai, J. G., Crews, T.E., DeHaan, L.R., Buckley, D.H., Ferris, H., Turner, R.E., Reynolds, H.L. & Wyse, D.L. 2010. Harvested perennial grasslands provide ecological benchmarks for agricultural sustainability. *Agriculture, Ecosystem and Environment*. 137: 3-12.

Glover, J.D., Reganold, J.P., Bell, L.W., Borevitz, J., Brummer, E.C., Buckler, E.S., Cox, C.M., Cox, T.S., Crews, T.E., Culman, S.W., DeHaan, L.R., Eriksson, D., Gill, B.S., Holland, J., Hu, F., Hulke, B.S., Ibrahim, A.M.H., Jackson, W., Jones, S.S., Murray, S.C., Paterson, A.H., Ploschuk, E., Sacks, E.J., Snapp, S., Tao, D., Van Tassel, D.L., Wade, L.J., Wyse, D.L. & Xu, Y. 2010. Increased food and ecosystem security via perennial grains. *Science*. 328: 1638-1639.

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M. & Toulmin, C. 2010. Food security: The challenge of feeding 9 billion people. *Science*. 327: 812-818.

Hayes, R.C., Newell, M.T., DeHaan, L.R., Murphy, K.M., Crane, S., Norton, M.R., Wade, L.J., Newberry, M., Fahim, M., Jones, S.S., Cox, T.S. & Larkin, P.J. 2012. Perennial cereal crops: An initial assessment of wheat derivatives. *Field Crops Research*. 133: 68–89.

Jaikumar, N.S., Snapp, S.S., Murphy, K. & Jones, S.S. 2012. Agronomic assessment of perennial wheat and perennial rye as cereal crops. *Agronomy Journal*. 104: 1716-26.

National Research Council. 2010. *Toward Sustainable Agricultural Systems in the 21st Century*. The National Academies Press, Washington, D.C.

Reganold, J.P. 2013. Comparing organic and conventional farming systems: metrics and research approaches. *Crop Management*. doi:10.1094/CM-2013-0429-01-RS.

Reganold, J.P., Jackson-Smith, D., Batie, S.S., Harwood, R.R., Kornegay, J.L., Bucks, D., Flora, C.B., Hanson, J.C., Jury, W.A., Meyer, D., Schumacher, Jr., A. Sehmsdorf, H., Shennan, C., Thrupp, L.A. & Willis, P. 2011. Transforming U.S. agriculture. *Science*. 332: 670-671.

Snapp, S.S., Blackie, M.J., Gilbert, R.A., Bezner-Kerr, R. & Kanyama-Phiri, G.Y. 2010. Biodiversity can support a greener revolution in Africa. *Proceedings of the National Academy of Sciences USA*. 107: 20840-20845.

Van Tassel, D.L., DeHaan, L.R. & Cox, T.S. 2010. Missing domesticated plant forms: can artificial selection fill the gap? *Evolutionary Applications*. 3: 434-452.

20

PERENNIAL GRAINS:
BEYOND BOOTLEGGING, FEASIBILITY AND PROOF-OF-CONCEPT

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ABSTRACT

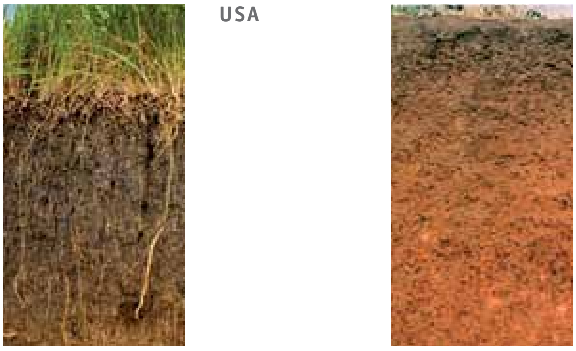
This paper examines the need for perennial grain development from a donor perspective. Why are perennial grains needed, especially in fragile and more remote areas? What kinds of investments does USAID currently make in perennial grain development? What lessons can be drawn to ensure the derivation of compelling cases for donor consideration? The paper concludes that: perennial grains must contribute to sustainable intensification and not extensification; must be demand-driven by scientists and farmers in target regions; priority targets should be identified based on priority regions and needs; with an emphasis on complementarity with current farming systems and research programmes; and short, medium and long-term benefits should be identified at different scales, to encourage staged investments.

Keywords: sustainable intensification, demand-driven, priority regions and needs, complementary systems, short, medium and long-term goals; staged investments

WHY PERENNIAL GRAINS

Food security is a major issue for the world. In the 1960s, the Green Revolution raised yields and grain supplies to defer the shortfall of food to a rising world population. Its successes, however, were not universal, as they focussed on narrow considerations of yield alone. High-yielding semi-dwarf cultivars were released which were responsive to alluvial and nutrient-rich soils, application of inputs, availability of irrigation. Consequently, benefits accrued in areas already productive, while those in more marginal situations of lower soil fertility and more remote locations generally missed out. For example, sub-Saharan Africa was largely bypassed, where 80 percent of soils have serious limitations, including low soil organic matter, short and variable wet seasons, and low if any investment in inputs.

FIGURE 1. CONTRASTING SOIL PROFILES IN USA (LEFT) AND SSA (RIGHT).



- >80% SOILS WITH SERIOUS LIMITATIONS
- LOW SOM
- 95% RAINFED
- HIGH VARIABILITY
- LOW INPUT INVESTMENT

Photo credit: Jim Richardson, Small World Gallery

Additional considerations needed to be taken into account, including broader considerations of yield (whole farm, livestock, nutrition), socio-economics (gender, cultural context) and natural resource management (soil, water, air, biodiversity). The likelihood is of further decline of the soil resource, with insufficient availability of organic matter (compost, manure, crop residues) to add to soil, leading to poor rainfall infiltration and retention as well as further decline and degradation of fragile soils. In this context, above-ground productivity will be low, with increased risk for investment in labour and inputs, and greater vulnerability to climate uncertainties. Farmer response is likely to require multiple planting operations, to address establishment and crop failure, requiring additional labour and necessitating lower yield potentials in the shorter seasons remaining. This may be partially compensated by extensification where additional land is available, but this in turn puts pressure on system sustainability via shorter or no fallow before the land is used again.

In this context, perennial grains could provide some relief by maintaining some stability of cover for land restoration, food and ecosystem security, nutrition and socio-economic considerations. For example, Dr Druba Thapa from the Nepal Agricultural Research Council sees potential for high-altitude perennial wheat on fragile soils in western Nepal "Perennial wheat may increase food and forage security significantly in the region, with deeper roots providing more stable grain and biomass yields. Deeper roots may increase uptake of selenium, zinc, iron and other minerals, and some of the 25 lines tested appear highly resistant to yellow rust."

FIGURE 2. HIGH ALTITUDE PERENNIAL WHEAT IN WESTERN NEPAL

Dr. Dhruba Thapa Nepal Agricultural Research Council Khumaltar Laitpur, Nepal

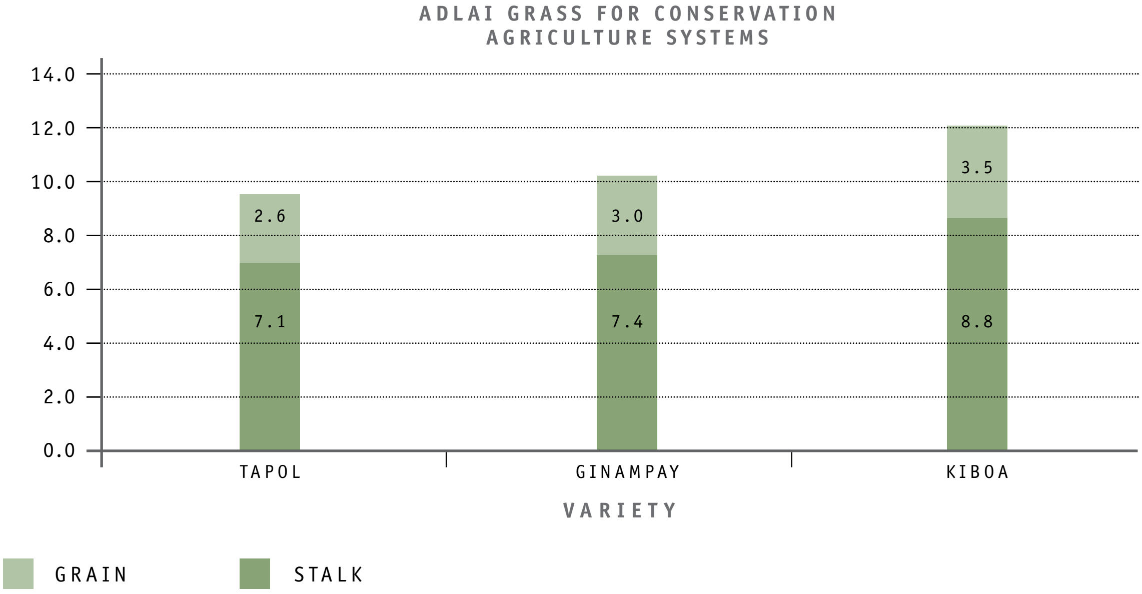
Photo credit: Dhruba Thapa

USAID PERENNIAL GRAIN INVESTMENTS

USAID is looking for opportunities where there is farmer demand for perennial systems that could assist their circumstances. These examples can be used to illustrate the criteria needed for priority to be assigned to such research investments.

Adlai grass has been identified for conservation agriculture systems in the Philippines. In diverse crop-livestock systems on acidic erodible hillsides, adlai grass has been shown to assist in providing a source of food, feed, resilience, ecosystem services complementary to farmer practice. Evidence is support of the concept is available from the World Agroforestry Centre and SANREM CRSP, where improved lines increased yield of both grain and stover.

FIGURE 3. ADLAI GRASS FOR CONSERVATION AGRICULTURE SYSTEMS. SANREM CRSP



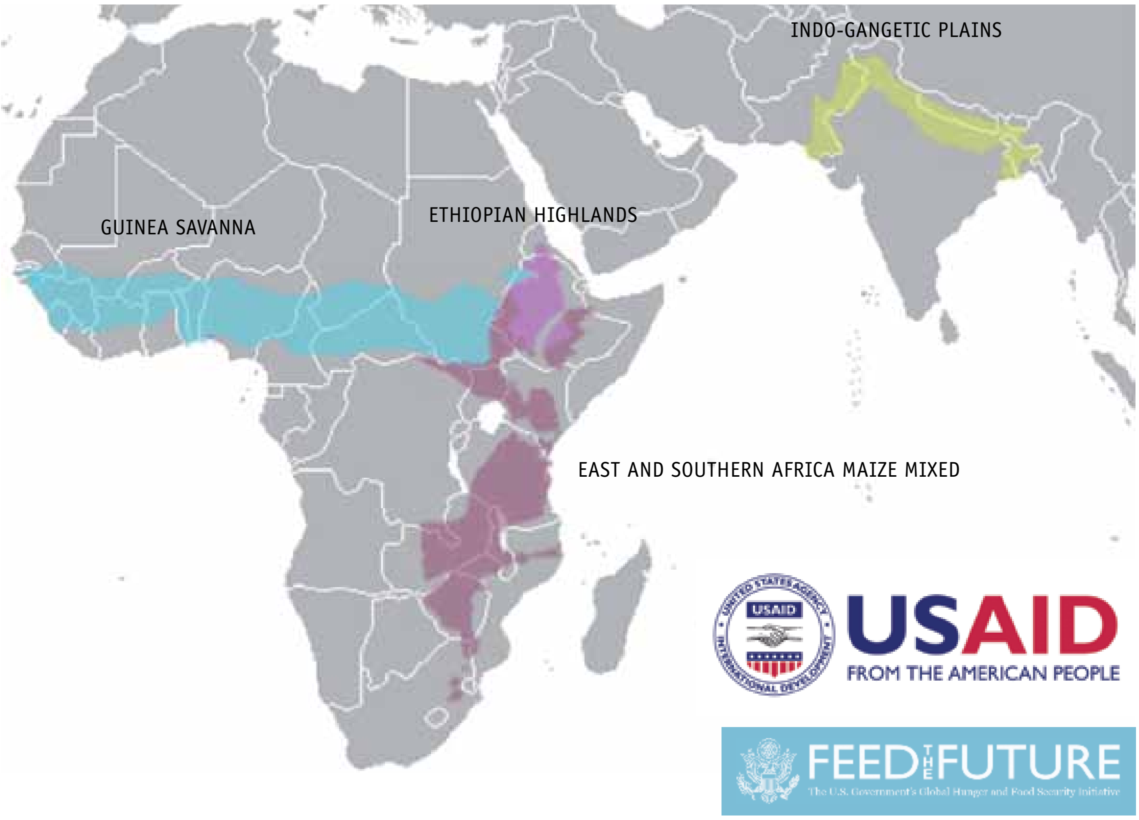
Likewise, the doubled-up legume systems presented by Snapp (this volume) allow diversification and intensification of traditional maize-dominated systems, using annual peanut, semi-perennial pigeon pea followed by maize, for substantial benefits in food, feed, resilience, ecosystem services, flexibility and complementarity with farmer practice. The system changes complement the role of fast maturing annuals along with the perennials.

FIGURE 4. INTERCROPPING OF PIGEON PEAS AND GROUNDNUT

Photo by Jim Richardson, Small World Gallery

A third example is the recent investment of US$5 000 000 over 5 years in the Feed the Future Innovation Lab for Climate Resilient Sorghum with University of Georgia and partners including West Africa and Ethiopia.

FIGURE 5. PROGRAMME FOR SUSTAINABLE INTENSIFICATION



Using these three case studies, it is possible to identify common features that encourage potential investment from a donor. These issues should be carefully considered by perennial grains researchers in proposing projects for donor support, noting that they require statements of interest and participation from target scientists and farmers in host countries.

ISSUES AND SOLUTIONS IN PERENNIAL GRAIN DEVELOPMENT FROM A DONOR PERSPECTIVE

1. Perennial grains are "supply-driven" solutions from developed country institutions

Issue: The development community seeks demand-driven solutions for targeted issues, regions, and farming systems. They can be identified by the international research community, national governments and farmers.

Solution: Constituency building is required: CGIAR, national research institutes, national governments, farmers.

FIGURE 6. PERENNIAL GRAIN BREEDING PROGRAMMES

Only the perennial wheat breeding programme in Nepal is located in a developing country that is of high-priority for development assistance (highlighted in green) for agencies like the United States Agency for International Development.

2. Focal crops and regions are based on scientist's interests and locations

Issue: No systematic analysis of highest-potential crops and regions, little overlap with high-priority regions already identified, and unclear how efforts will fit into or impact high priority farming systems.

Solution: Global survey and screening of high potential crops, "What is out there?" Opportunity analysis of high priority regions, crops, socio-economic conditions, "What is needed?" Modelling and early field trials in priority regions, "What is possible?"

3. Discussion remains focussed on annual vs perennial (or organic vs conventional, or polyculture vs monoculture)

Issue: "Either - or", "instead of" and "replace" narrow the possibilities, and suggest rotations are not possible. Annuals are and should be here to stay. Such language increases anxiety of all but full proponents, and blurs into "Low input vs Industrial systems".

Solution: "Both" and "Complementary" open up opportunities. "Perenniation," the integration of perennials into annual-based systems, with "complementary parallel breeding" and "Complementary parallel management".

4. Too much bootlegging; too little leveraging and coordination

Issue: Individual projects are not fully benefiting from other efforts. Information transfer is slow. Cost is presented in years; as time is required. Impairs production of international public good.

Solution: (This has equal responsibility with donors). Each programme needs to emphasise communication and coordination with the global community (beyond specific crops). Develop a professional society with formal lines of communication. Establish a World Perennial Grain Research Centre.

Use the perennial grains blog at Michigan State University for resources and announcements, pwheat.anr.msu.edu/index.php/about/, e.g. "Polyculture and Perennial Grains for Sustainable Agriculture" Symposium at the ASA-CSSA-SSA Annual Meetings in Tampa Florida in November 2013; e.g. Special Symposium "Perennial Grains for Food Security in a Changing World: Gene to Farm Innovations" at the AAAS Annual Meetings in February 2014.

CONCLUSIONS

To attract sufficient support for perennial grain research and development, we must better understand and communicate the multiple sustainability benefits of perennial grain systems, especially in the area of climate change adaptation and mitigation. The greater use of websites, blogs (e.g. the Michigan State University blog), professional conferences, and webinars can help communicate the scientific results to wider audiences, including scientific funding agencies and policymakers. Current efforts lack effective coordination and leveraging. A more formal framework for communicating information, transferring research results and germplasm, and identifying priorities needs to be developed via organized international organizations and professional societies.

To specifically address the needs of developing countries, particularly those regions most in immediate need of increased food and ecosystem security, perennial grain advocates should address additional issues as follows:

1. Perennial grains must contribute to sustainable intensification and not lead to extensification, which would require more land for production of the same amount of food;
2. Perennial grain development for international development contexts must include scientists, policymakers, and farmers in target regions. Currently, efforts are primarily ‘demand-driven’ by scientists working in developed countries (Figure 6);
3. The crops and regions on which current efforts are focused are primarily based on scientist interests and locations, not on systematic analyses of priority regions and needs. Computer modelling combined with on-the-ground trials can help identify priority farming systems and crops on which to focus limited resources;
4. Much of the discussion about perennial grains to date has been on the costs or benefits of annual crops versus perennial crops; most farmers in target priority regions rely on both. Greater attention must be paid to complementarity of annual and perennial crops and systems, from complementarity in plant breeding programmes to complementarity at the farm management level;
5. The timeline for widespread impact of perennial grains is long for traditional development funding streams. While some funding has been directed toward long term, high risk, high reward projects (“blue sky” projects), there are potential short and medium term benefits derived from investigating and/or developing perennial grains at multiple scales.

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A NEW SPECIES OF WHEAT THAT CONTINUES TO GROW AFTER HARVEST

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ABSTRACT

Crosses with hexaploid wheat and intermediate wheat grasses (*Thinopyrum intermedium*) were used to develop perennial wheat that exhibits post sexual cycle regrowth. These lines were bred to senesce fully after seed development and then regrown after a dormant cycle. Some plants however exhibited continuous growth in areas with mild winters and wet autumn months such as the Pacific Northwest areas of Washington State in the United States. Plants with continuous growth were at first discarded but are now being selected as a possible forage and grain multi-use crop for animal production. Forage quality is as high as wheat hay but the tonnage per acre is much greater. The chromosome constitution of the lines are stable at 56 chromosomes. Forty two are wheat and the other 14 are at this point unidentified. There are awned and awnless types and seed colour is red, white or blue. Height can exceed 2 metres. These lines seem to have great promise in short term rotations (2 to 3 years) where large amounts of organic matter is needed and flexibility on end-uses ranging from straw to hay to grain is desired.

Keywords: wheat, *Thinopyrum intermedium*, awn, Salish Blue

Beginning in 1995, crosses with hexaploid wheat and intermediate wheat grasses (*Thinopyrum intermedium,* -Host- Barkworth & Dewey) were used to develop perennial wheat that exhibits post-sexual cycle regrowth. These lines were bred to senesce fully after seed development and then regrow after a dormant cycle. Some plants however exhibited continuous growth in areas with mild winters and wet autumn months such as the Pacific Northwest areas of Washington State in the United States. Plants with continuous growth were at first discarded but are now being selected as a possible forage and grain multi-use crop for animal production. We have named an exemplary breeding line from this population "Salish Blue." Salish Blue is an awnless, blue-seeded derivative of these breeding efforts. Forage quality is as high as wheat hay but the tonnage per acre is much greater. The chromosome constitutions of the lines are stable at 56 chromosomes. Forty two are wheat and the other 14 are at this point *Th. intermedium* of unidentified homoeology groups. There are awned and awnless types and seed color is red, white or blue. Height can exceed 2 metres. These lines seem to have great promise in short term rotations (2 to 3 years) where a large amount of organic matter is needed and flexibility on end-uses ranging from straw to hay to grain are desired.

In situ Hybridization

We performed fluorescent genomic *in situ* hybridization (FGISH) on root tip cells from Salish Blue using biotinylated genomic DNA from *Thinopyrum ponticum* Barkworth and Dewey as a probe. gDNA of *Th. ponticum* was used because our previous studies indicated that the 10n *Th. ponticum* genome is derived from each of the principal diploid ancestral genomes for all of the *Thinopyrum* species and thus is an ideal all-purpose probe for detecting *Thinopyrum* chromatin (Arterburn *et al.* 2011). Signal detection was accomplished using avidin-fluorescein and biotinylated anti-avidin. The FGISH probe bound strongly to the alien chromosomes, even compared to positive controls (metaphase cells of the *Thinopyrum* amphiploid AgCS). Fluorescent signals clearly indicate that 14 of the 56 chromosomes of Salish Blue are of alien origin, and the efficacy of probe binding indicates a member of the *Thinopyrum* species as the alien donor (Figure 1). Six replicates produced identical results, suggesting that Salish Blue is stable at 56 chromosomes. Because the wild parent of Salish Blue is the hexaploid *Th. intermedium*, FGISH is insufficient to determine which specific chromosomes of the parent have been retained in this amphiploid and which were lost during backcross breeding efforts. Because our previous investigations have confirmed that *Th. intermedium* is a descendant of *Th. elongatum* (2n = 14, EE), *Th. bessarabicum* (2n =14, JJ) and *Pseudoroegneria spicata* (2n = 14, StSt), we sought a means to use DNA evidence to indicate which specific homoeologous pairs from these donor genomes are present in Salish Blue (Arterburn *et al.* 2011).

MARKER ANALYSIS

We sought to identify polymorphisms in Salish Blue that correspond to known polymorphic loci on specific chromosomes from the E, J or St genomes. To accomplish this, we analyzed 24 DNA markers that have been localized to specific chromosomes in those diploid *Thinopyrum* species that are related to likely alien chromosome donors of Salish Blue (e.g. *Thinopyrum intermedium*). The markers analyzed were a combination of SSR polymorphisms detected on chromosomes of the E genome of *Th. elongatum*, and cleaved amplified polymorphic sequence (CAPS) polymorphisms detected on chromosomes of the St genome of *Ps. spicata* (Hu *et al.* 2012; Mullan *et al.* 2005). There are a further 20 SSR and CAPS markers available that we intend to assay. The results of this marker analysis can be seen in Table 1.

During this analysis, we identified five polymorphisms specific to Salish Blue. Curiously, only two of these amplicon/fragment size polymorphisms matched with a putative alien donor. A 315 bp polymorphism, amplified by SSR primers associated with chromosome 1E is shared between Salish Blue and the hexaploid *Th. junceum*. A 435 bp polymorphism, amplified with SSR primers associated with chromosome 3E, is shared between Salish Blue and the diploids *Th. elongatum* and *Th. bessarabicum*. While not conclusive evidence on its own, this suggests that two alien pairs in Salish Blue belong to homoeology groups 1 and 3 respectively. Two other polymorphisms detected in Salish Blue were amplified by primers associated with chromosome 7E, although the fragment sizes were subtly different from those detected in *Th. elongatum*, possibly due to additional microsatellite expansion in Salish Blue. A restriction cut-site polymorphism was detected in Salish Blue for a CAPS marker associated with chromosome 1St, although the fragment generated was distinct from the polymorphism associated with *Ps. spicata*.

This preliminary evidence indicates that alien chromosomes of homoeology groups 1, 3 and 7 may be present in Salish Blue. Additional marker and sequence work will be able to confirm this as well as elucidate the origins of the remaining four chromosomes pairs present in this line.

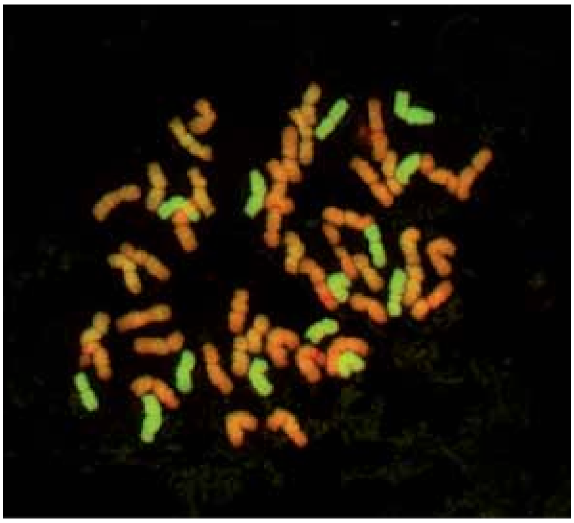
NUCLEAR GENE SEQUENCING EFFORTS

To provide further evidence of *Th. intermedium* chromosomes in Salish Blue, we are in the process of cloning and sequencing the various alleles of the beta-amylase I (bmyI) gene and the granule-bound starch-synthase (GBSSI) gene present in this amphiploid line. We have used this method successfully in the past to detect genome origins and have identified specific polymorphisms associated with distinct *Th. intermedium* (Arterburn *et al.* 2011). Identification of bmyI and GBSSI alleles matching those found in *Th. intermedium* will also confirm the presence of alien homoeology groups 4 and 7, respectively, in Salish Blue. This method is work-intensive in amphiploid samples such as Salish Blue because it requires sequencing of many clones from multiple PCR products to ensure that all alleles are detectable and free of background heterogeneous signal.

CONCLUSION

The genomic origin of the additional 14 chromosomes will lead to the naming of a new species of wheat. This new species and improved varieties within this species will have value in perennial wheat breeding programmes. Identification of the chromosomes will also lead to more efficient mapping and tagging of genes that control traits of interest such as regrowth and stay-green.

FIGURE 1. FLUORESCENT GENOMIC *IN SITU* HYBRIDIZATION (FGISH) OF SALISH BLUE



Identification of the chromosomes will also lead to more efficient mapping and tagging of genes controlling traits of perenniality in crops

TABLE 1. SUMMARY OF SSR AND CAPS MARKER ANALYSIS. MARKERS WHICH DETECTED NO *THINOPYRUM* POLYMORPHISMS ARE EXCLUDED

MARKER NAME	CHROMOSOME LOCATION	MARKER TYPE	POLYMORPHISMS DETECTED IN OUR STUDY
MWG634	4ES	STS	*Th. elongatum* = 450 bp
			Salish Blue = No polymorphic band
Xedm17	1E	SSR	*Th. elongatum* = 250 bp
Xedm28	2ES	SSR	*Th. bessarabicum* = 200 bp
			Th. elongatum = 200 bp

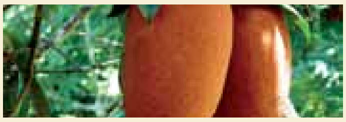
MARKER NAME	CHROMOSOME LOCATION	MARKER TYPE	POLYMORPHISMS DETECTED IN OUR STUDY
Xedm54	5ES	SSR	*Th. elongatum* = 185 bp
			Th. elongatum = 185 bp
			Salish Blue = No polymorphic band
Xedm74	1EL	SSR	*Th. bessarabicum* = 325 bp and 285 bp
			***Th. junceum* = 315 bp and 285**
			Salish Blue = 315 bp
			Th. elongatum = 275 bp
Xedm105	7EL	SSR	*Th. elongatum* = No polymorphic band
			Th. bessarabicum = No polymorphic band
			Salish Blue = 340 bp
Xedm109	3E	SSR	***Th. elongatum* = 435 bp**
			***Th. bessarabicum* = 435 bp**
			Salish Blue = 435 bp
Xedm149	6EL	SSR	*Th. elongatum* = 175 bp
Xedm156	7ES	SSR	*Th. elongatum* = 260 bp
			Th. bessarabicum = 270 bp and 295 bp
			Salish Blue = 280 bp
TNAC1001	1St	CAPS	Salish Blue = 275 bp
TNAC1102	2St	CAPS	*Th. bessarabicum* = 975 bp
			Th. junceum = 975 bp
			Th. intermedium = 1 000 bp
			Salish Blue = No polymorphic band
TNAC1178	2St	CAPS	*Th. bessarabicum* = 900 bp
			Th. intermedium = 900 bp
			Salish Blue = No polymorphic band
TNAC1248	3St	CAPS	*Th. elongatum* = 800 bp
			Th. intermedium = 750 bp
			Salish Blue = No polymorphic band
TNAC1408	4St	CAPS	*Th. intermedium* = 700 bp
			Salish Blue = No polymorphic band
TNAC1485	5St	CAPS	*Th. elongatum* = 1 000 bp
			Th. bessarabicum = 640 bp
			Th. intermedium = 640 bp
			Salish Blue = No polymorphic band
TNAC1674	6St	CAPS	*Th. elongatum* = 550 bp
			Th. bessarabicum = 775 bp
			Th. intermedium = 525 bp
			Salish Blue = No polymorphic band

REFERENCES

Arterburn, M., Kleinhofs, A., Murray, T. & Jones, S. 2011. Polymorphic nuclear gene sequences indicate a novel genome donor in the polyploid genus *Thinopyrum*. *Hereditas*. 148: 8-27.

Hu, L., Li, G., Zhan, H., Liu, C. & Yang, Z. 2012 New St-chromosome-specific molecular markers for identifying wheat–*Thinopyrum intermedium* derivative lines. *Journal of Genetics*. 91: e69–e74.

Mullan, D., Platteter, A., Teakel, N., Appels, R., Colmer, T., Anderson, J. & Francki, M. 2005. EST-derived SSR markers from defined regions of the wheat genome to identify Lophopyrum elongatum specific loci. *Genome*. 48(5): 811-822.



22 Twelve principles for better food and more food from mature perennial agroecosystems

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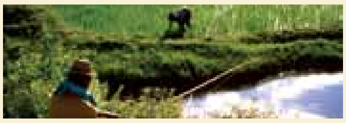

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POLICY, ECONOMICS AND WAY FORWARD

22

TWELVE PRINCIPLES FOR BETTER FOOD AND MORE FOOD FROM MATURE PERENNIAL AGROECOSYSTEMS

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ABSTRACT

An analysis of the factors leading to unsustainable agriculture and its associated problems of food insecurity, malnutrition and poverty, identifies a downward spiral of land degradation and social deprivation which is associated with lower crop yields, loss of biodiversity and agro-ecological function, and declining farmer livelihoods. This spiral is responsible for the Yield Gaps (the difference between the potential yield of a modern crop varieties and the yield actually achieved by farmers) found in many modern farming systems. To reverse this complex downward cycle and close the Yield Gap requires simultaneous crop and soil husbandry, ecological and socio-economic interventions at several different 'pressure-points' within this spiral. This paper advocates 12 important principles for the achievement of food security, which including the adoption of a simple, yet highly adaptable, three-step generic model involving perennial crops to kick-start the reversal of the spiral and so the closure of the Yield Gap. This agroforestry approach involves both the use of biological nitrogen fixation from trees and shrubs, as well as the participatory domestication and marketing of new highly nutritious cash crops derived from the indigenous tree species that provide poor people with the traditionally and culturally important foods, medicines and other products of day-to-day importance. Closing the Yield Gap improves food security by improving the yields of staple crops, but also has beneficial social, economic and environmental impacts. Agroforestry involving the combination of many annual and perennial crop species is, therefore, not an alternative to current agricultural systems, but is a way to diversify and enrich them, making them more sustainable. It does this by increasing food and nutrition security, increasing social and environmental sustainability, generating income, creating business and employment opportunities in rural communities and mitigating climate change. Agricultural policy currently tends not to appreciate these outcomes delivered by tropical and sub-tropical production systems which are based on perennial species and meet the requirements of 'sustainable intensification'.

Keywords: agroforestry, land degradation, tree domestication, poverty, sustainable intensification, yield gap

INTRODUCTION

Agriculture faces a very complex set of social and biophysical issues associated with the economic, social and environmental sustainability. This paper examines the role of perennial species, especially trees, in the attainment of improved staple crop yields; provision of nutritious traditional food; the reduction of poverty, hunger, malnutrition and environmental degradation; the improvement of rural livelihoods; as well as the mitigation of climate change - all with increased economic growth with a programme of Integrated Rural Development (Leakey, 2010; 2012a/b). It therefore provides a model, or policy roadmap, for the delivery of the sustainable intensification of productive tropical and sub-tropical agriculture which is pro-poor and multifunctional – i.e. enhancing agriculture economically, socially and environmentally (Leakey, 2012a). This paper is based on 12 interconnected Principles (Box 1).

BOX 1. TWELVE PRINCIPLES FOR IMPROVED FOOD SECURITY WITHIN MULTIFUNCTIONAL AGRICULTURE AND ENHANCED RURAL DEVELOPMENT

	PRINCIPLES
1	Ask, do not tell
2	Do not throw money at farmers, but provide skills and understanding
3	Build on local culture, tradition and markets
4	Use appropriate technology, encourage diversity and indigenous perennial species
5	Encourage species and genetic diversity
6	Encourage gender/age equity
7	Encourage farmer-to-farmer dissemination
8	Promote new business and employment opportunities
9	Understand and solve underlying problems: The Big Picture
10	Rehabilitate degraded land and reverse social deprivation: Close the 'Yield Gap'
11	Promote 'Multi-functional Agriculture' for environmental/social/economic sustainability and relief of hunger, malnutrition, poverty and climate change
12	Encourage Integrated Rural Development

PRINCIPLES

PRINCIPLE 1. Ask farmers what they want, do not tell them what they should do.

As the human population has grown, shifting cultivation has become less and less sustainable as deforestation has made new productive land scarcer. One consequence of this has been that farmers have been forced to become more sedentary. With this their crop yields have declined and farmers have struggled to feed their families, let alone generate income from surplus production. These families have therefore becoming increasingly trapped in hunger, malnutrition and poverty and are in need of help and substantial policy reform to free them from the circumstances that they are in. The problem originates with the advent of colonialism and the industrial revolution, because there has been a tendency for leaders in developed countries to think that agricultural developments that have worked in the temperate zone must be applicable in the tropics; despite big differences in the climate, soils, ecology and socio-economic conditions. As a result agricultural policy in developing countries has often been based on a model that is not well adapted to local conditions.

Recognizing the above issue, the work reported here began with a participatory approach to priority setting (Franzel *et al.* 1996; 2008) that sought the ideas of farmers on what they needed. These farmers identified their desire to grow the forest species from which, as hunter gatherers and subsistence farmers, they had formerly gathered wild fruits, nuts and other products of everyday value (Leakey, 2012a). This has led to an unconventional approach to agricultural development that focuses on the domestication of indigenous fruit and nut trees using a participatory approach. From this initiative the following principles have emerged (Tchoundjeu *et al.* 2002; 2006; 2010; Leakey *et al.* 2003; Asaah *et al.* 2011; Degrande *et al.* 2006; Leakey and Asaah, 2013).

PRINCIPLE 2. **Provide appropriate skills and understanding, not unsustainable infrastructure.**

Many agricultural and other rural development projects provide funding for communities to implement new and 'improved' technologies – often ones based on concepts which are 'foreign' to the farmers. While the funds are flowing these projects can be successful, but very often when the project comes to an end the new approaches are not sustained. Typically this is because the stakeholders are still dependent on a continuing stream of finance, but this is often exacerbated by a lack of 'buy-in' to the new approach. To try to overcome these problems the work reported here first asked farmers what they wanted and then, once that was agreed, went on to assist by providing skills and understanding through training, but without direct financial assistance. Thus project funds were spent on training and mentoring the participating communities with only the provision of minimal facilities. Then, as the concepts were adopted and the programme grew, these facilities were improved by both donor funds and by community contributions. In this way, pilot village nurseries grew into Rural Resource Centres staffed by village members with support from local NGOs and Community Based Organizations (CBOs) (Tchoundjeu *et al.* 2006, 2010; Asaah *et al.* 2011). This has been found to be an effective strategy for the dissemination of agroforestry innovations (Degrande *et al.* 2012).

PRINCIPLE 3. **Build on local culture, tradition and markets.**

In the past, tree products were gathered from natural forests and woodlands to meet the everyday needs of people living a subsistence lifestyle. Non-timber forest products gathered from the wild in this way have played an important role in the lives and culture of local people, as is recognized by the study of local flora (e.g. Abbiw, 1990) and ethno botany (Cunningham, 2001) With the application of intensive modern farming systems this resource has declined. To rebuild and improve this useful resource the concept of tree domestication for agroforestry was proposed in 1992 (Leakey and Newton, 1994) and subsequently implemented by the World Agroforestry Centre (ICRAF) as a global initiative from 1994 (Simons, 1996). Great progress has been made in the first two decades of this initiative (Leakey *et al.* 2005; 2012) which have encouraged local entrepreneurism in the processing and marketing of agroforestry tree products. This has had beneficial impacts on farmers' livelihoods (Tchoundjeu *et al.* 2010; Leakey, in press a).

To capitalize on this tradition and culture, the domestication of indigenous fruit and nut trees for integration into farming systems through agroforestry is based on participatory processes involving local communities. The prime objective of the participatory approach is to involve the target communities in all aspects of the planning and implementation of the programme so that they have ownership of the programme, while also benefitting from the close involvement of researchers and NGOs as mentors in the domestication programme. By building on tradition and culture in this way, participatory tree domestication has stimulated rapid adoption by growers and has enhanced the livelihoods of the households and communities involved (Leakey *et al.* 2003; Simons and Leakey, 2004; Asaah *et al.* 2011).

In implementing this strategy it is of great importance to recognize the legal and socially-important communal rights of local people to their traditional knowledge and local germplasm (Lombard and Leakey, 2010) and to ensure that they benefit from their use and are rewarded for sharing them for the wider good. Because of the sensitivity arising from past commercial exploitation of these rights by individuals, companies, academics, international agencies and government, it is very clear that the partners in domestication programmes have to earn the trust of local communities. This is to ensure that benefits flow back to the farmers and communities, the recipients of traditional knowledge and germplasm should enter into formal 'Access and Benefit Sharing' agreements (ICRAF 2012) in which the rights of the holders of knowledge and genetic resources will be legally recognised.

With poverty alleviation as one of the objectives of the domestication of indigenous trees it is clear that incentives for, and approaches to income generation are important in the overall strategy. Consequently, improving and expanding the markets for agroforestry trees and their products are central to the strategy. The experience of the last 10-15 years indicates that this is transforming the lives of the participating farmers and helping them to break-into new business and employment opportunities (Leakey and Asaah, 2013).

In many countries land tenure systems are complex with a combination of community customary rights and individual legal rights based on land purchase. In addition, government attempts to regulate logging and deforestation make the sale of tree products illegal. These issues can affect farmers' decisions about the growth of tree crops. In Cameroon, a study of formal policies found that regulations do not clearly distinguish between products from trees found in the wild and those gathered from farmers' fields (Foundjem-Tita *et al.* 2012). This finding supports the need to distinguish between common-property wild forest resources (e.g. non-timber/wood forest products) and private domesticated tree resources (agroforestry tree products) growing in farmland (Simons and Leakey, 2004) and to recognise that the exploitation, transport, import and export of indigenous fruit crops from farmers' fields do not pose any threat to conservation (Schreckenberg *et al.* 2006b). Defining agroforestry tree products (timber and non-timber) as conventional farm products in this way should increase farmers' incentives to formally cultivate trees and harvest their products, with beneficial impacts on farmers' income, national revenues, rehabilitation of degraded land and the environment (Schreckenberg *et al.* 2006a).

A strategy to increase income generation from the sale of tree products in local markets is particularly important as local people are familiar with the use of these food and medicinal products and the demand typically exceeds supply. In the longer term, this trade often has potential to expand regionally and even internationally as the products become more widely known or better processed for global customers. However, as the commercialization process involves more players and becomes more complex, so the risks that producers will be exploited increases. To counter this risk, innovative approaches to ensure that farmers and local communities are rewarded for their marketing innovations have been developed by PhytoTrade Africa and are being extended to tree domestication (Lombard and Leakey, 2010; Leakey, in press a). Again, the approach involves working with indigenous communities and helping them to secure long-term access to markets in ways which reward them and protect their intellectual property rights.

PRINCIPLE 4. **Use appropriate technology and indigenous perennial species.**

Principles 1 and 3 mentioned the relevance of indigenous trees and their products to tropical and sub-tropical farmers. To capture, harness and improve the flow of benefits from these trees recent approaches to their domestication have focussed on the large opportunity for genetic selection and clonal propagation as horticultural cultivars. This is based on the capacity of vegetative propagation to capture and fix desirable traits, or combinations of traits, found in individual trees (Leakey and Simons, 2000). This approach to clonal propagation also has the benefit that selected trees can be propagated from mature tissues so that the cultivar has a lower physical stature and early fruiting - making early returns on effort and the harvesting of fruits easier.

The simplest technique for mass clonal propagation is the rooting of leafy stem cuttings. Studies over the last 50 years have greatly enhanced the understanding of basic principles for robust and efficient techniques (Leakey, 2004; in press b), as well as the development of simple, low-cost propagation systems for implementation in remote village nurseries without access to running water and electricity (Leakey *et al.* 1990). With only a little training, these propagators made from locally available materials have been widely and successfully adopted around the tropics by unskilled and illiterate farmers and have opened up the opportunity to develop improved clones/cultivars of over 50 tree species for local planting, as well as for sale to others. Without this appropriate technology participatory tree domestication would probably not have been possible.

To decide which trees have potential for cultivar development it is necessary to have an understanding of the tree-to-tree variation within wild populations. Fortunately farmers who have gathered products from the wild trees in their area are generally well aware which trees have particular traits, such as large fruit or nut size, good taste, or particular elements of seasonality – all desirable traits that attract a good market price (Figure 1). To assist this process of farmer selection, appropriate quantitative techniques have also been developed for the selection of superior trees that meet the needs of local markets and industries. The tree-to-tree variation in hundreds of morphological traits of importance to the development of food, cosmetic, pharmaceutical and other products have been assessed in the field and used to identify appropriate multi-trait combinations that can be easily understood by local farmers. Scientific studies of chemical and physical traits have been done in parallel and the results of these are used to assist farmers to understand the potential for the development of new commercial products. The above scientific inputs to the understanding of genetic variation can then inform the process of farmer selection and help to provide guidance of how best to meet the needs of different market opportunities. Based on the concept of 'ideotypes' for tree selection (Leakey and Page, 2006) cultivars can be developed that have the ideal combination of traits for a product to meet the needs of a particular market. So, for example an ideotype for a fresh fruit would have a lot of flesh (and small seeds/nuts/kernels), be sweet, juicy, tasty, nutritious and look attractive. On the other hand, a nut ideotype would have a large kernel(s) (and probably little flesh), have a thin shell so that it is easily cracked, be rich in edible oil with an appropriate fatty acid profile or have other characteristics meeting the needs of the cosmetic or pharmaceutical industries. In both instances, these quality traits are ideally associated with a high yield of fruits or nuts, so that the cultivar can be said to have a high 'harvest index' – a large amount of 'ideal' harvestable product.

FIGURE 1. FRUITS OF SAFOU (*DACRYODES EDULIS*) FROM A MARKET IN YAOUNDÉ IN CAMEROON, WITH THEIR ASSOCIATED PRICE WHICH RECOGNIZES BOTH SIZE AND FLAVOUR

***c.f.* three fruits selling for 250CFA *versus* 22 fruits selling for 50CFA.**

To assist the marketing of tree products (especially nuts), simple, low-technology tools are being developed for nut cracking and the pressing of oil from nut kernels (e.g. Mbosso *et al.* in press). These are labour saving, better for large scale processing and safer than many tradition methods, such as the use of a machete to extract kernels.

PRINCIPLE 5. **Encourage species and genetic diversity.**

Of the 20 000 plant species producing edible products only about 0.5 percent have been domesticated as food crops, yet many have the potential to become new crops through the implementation of participatory domestication; indeed research is already in progress in over 50 tree species (Leakey *et al.* 2012). Adding new crops to small farms reduces risks from crop and market failures, as well as playing an important role in the re-building of agro-ecological functions on degraded farm land (Leakey, 1999b; 2012a). In environmental terms, the diversification with long-lived perennial plants is important because it is the way to rebuild the ecological functions of agro-ecosystems and landscapes.

Some people are rightly concerned that the domestication of new food crops will result in the loss of their genetic diversity by narrowing the genetic base. This can certainly happen if the domestication process is not based on a wise strategy that is correctly implemented. In the case of agroforestry trees being domesticated by participatory processes implemented at the village level, there is good evidence that both the strategy (Leakey and Akinnifesi, 2008) and the implementation (Pauku *et al.* 2010) are not creating any serious concerns. About 70-80 percent of the tree-to-tree variation is found at the village level and selected trees with morphologically desirable traits have been found by DNA analysis to be unrelated. Consequently, development of different sets of unrelated cultivars in different villages ensures that the narrowing of the genetic base is minimal. In other words “decentralized domestication” seems to be a means of ensuring genetic diversity is retained.

Furthermore, by gaining an understanding of the tree-to-tree variation and developing different sets of cultivars based on ideotypes formulated to meet the needs of different markets it should be possible to repackage genetic diversity and develop cultivars which are as different from each other as breeds of dogs are different from each other (Leakey, 2012a), without destroying the wild species.

In the scientific approach to selection, modern laboratory techniques are being increasingly used to examine traits which are not visible to the naked eye. For example, to quantify genetic variation in the chemical and physical composition of marketable products such as polysaccharide food thickening agents, nutritional content (protein, carbohydrate, oils, fibre, vitamins and minerals, etc.) by proximate analysis, medicinal factors like anti-inflammatory properties, the composition of essential oils and fatty acids, the determination of wood density, strength, shrinkage, colour, calorific value and other important wood properties correlated with tree growth (Leakey *et al.* 2012). Molecular DNA analysis is increasingly being used to gain understanding of genetic variation and relatedness (Jamnadass *et al.* 2009).

PRINCIPLE 6. **Encourage gender and age equity.**

In many rural communities around the world, women in particular have been engaged in gathering, using and marketing tree products. One of the purposes of a participatory tree domestication strategy is to ensure that all members of the community, whether male or female, are empowered by the programme and the beneficiaries of the outputs of their own initiatives and labour. This has been found to enhance the livelihoods of the community members in general and promote social and gender equity (Kiptot and Franzel, 2012), with exciting long-term benefits for youths (Leakey and Asaah, 2013; Degrande *et al.* 2012).

PRINCIPLE 7. **Encourage farmer-to-farmer dissemination.**

Through the development of Rural Resource Centres as the hubs of participatory tree domestication there has been a steady growth in the number of communities (from two to over 450) and number of people (from 20 to over 10 000) becoming engaged in participatory tree domestication as satellite nurseries have been developed in the areas around the Rural Resource Centres (Tchoundjeu *et al.* 2006) - a process which in continually expanding (Asaah *et al.* 2011). Much of this has been word-of-mouth neighbour-to-neighbour dissemination, but in addition efforts have been made for longer distance dissemination by community-to-community visits, fairs and competitions, as well as stories in the national media.

Evidence from Cameroon (Degrande *et al.* 2012) suggests that the involvement of grassroots organizations in the extension of agroforestry through the Rural Resource Centres has led to a relatively high level of satisfied farmers and been successful in reaching the women and youths often excluded by other extension systems.

PRINCIPLE 8. **Promote new business and employment opportunities.**

As mentioned earlier, local markets often exist for traditionally important food and non-food products from trees. Thus local knowledge and acceptance of the products is good. Again as mentioned, through the application of the ‘ideotype’ concept (Leakey and Page, 2006), tree domestication enhances the quality, uniformity and marketability of these products as clonal cultivars, selected for commercially desirable traits, stimulate a quantum leap in the marketability of the products. This means that traders and wholesalers can purchase a large volume of uniform, high quality product from a recognized and named cultivar. In return, hopefully the producer will receive a higher price, as it is clear that consumers are willing to pay more for the more desirable varieties. To ensure that these price benefits are passed back to the small-scale community producers, the development of trade associations, business partnerships and agreements are essential (Lombard and Leakey, 2010). Interestingly the benefits from tree domestication become increasingly important as the value chain progresses from local to global (Leakey and van Damme, in press). In the case of marketing Njangsang (*Ricinodendron heudelottii*) kernels in Cameroon more kernels were traded, with faster integration and greater financial benefits when interventions to enhance commercialization were implemented (Cosyns *et al.* 2011). Other relevant evidence from Cameroon suggests that the adoption of collective action in kola nut production is influenced by its ease of use, absence of entry barriers and emphasis on social activities which serve as an intrinsic motivator for farmers (Gyau *et al.* 2012).

Much work remains to be done to select cultivars for year-round production and to develop post-harvest technologies for the extension of the shelf life of agroforestry tree products and processing for added value. Interestingly, there are a growing number of processed tree products on regional and international markets – for example there are over 410 Baobab products (PhytoTrade Africa, www.phytotradeafrica.org). Many of these products rely on wild harvesting for their supply; this supply can be of very variable (non-uniform) and of mixed quality, as well as irregular across seasons and producers.

With the increasing importance of market acceptability, exclusivity and distinctiveness the use of ideotypes for the identification of the specific trait combinations become more and more critical. To meet this demand increasingly sophisticated research to determine the genetic variation in the chemical, physical and medicinal properties of the raw products is underway (Leakey *et al.* 2012). This also leads to the need for stronger linkages between agroforestry researchers and partners in industry (Leakey, 1999a), as can be seen in the case of Allanblackia oil (Jamnadass *et al.* 2010).

PRINCIPLE 9. **Understand and solve underlying problems – the Big Picture.**

Over the last 60 years, agricultural intensification has resulted in substantial gains in crop and livestock production. These are due to advances in breeding (e.g. genetic gain, stress resistance), husbandry (e.g. fertilizer, irrigation, mechanization), policy (e.g. Intellectual Property Rights, variety release processes), microfinance (e.g. credit, provision of inputs), education and communication (e.g. farmer-field schools), and market and trade (e.g. demand, incentives). World cereal production, for example, has more than doubled since 1961, with average yields per hectare also increasing around 150 percent (with the notable exception of sub-Saharan Africa). Likewise, modern agriculture has led to great improvements in the economic growth of many developed countries, with concomitant improvement in the livelihoods of many farmers. In real terms, food has become cheaper (although currently prices are increasing) and calorie and protein consumption have increased. Thus, on a global scale, the proportion of people living in countries with an average per capita intake of less than 2200 kcal per day has dropped from 57 percent in the mid-1960s to 10 percent by the late 1990s.

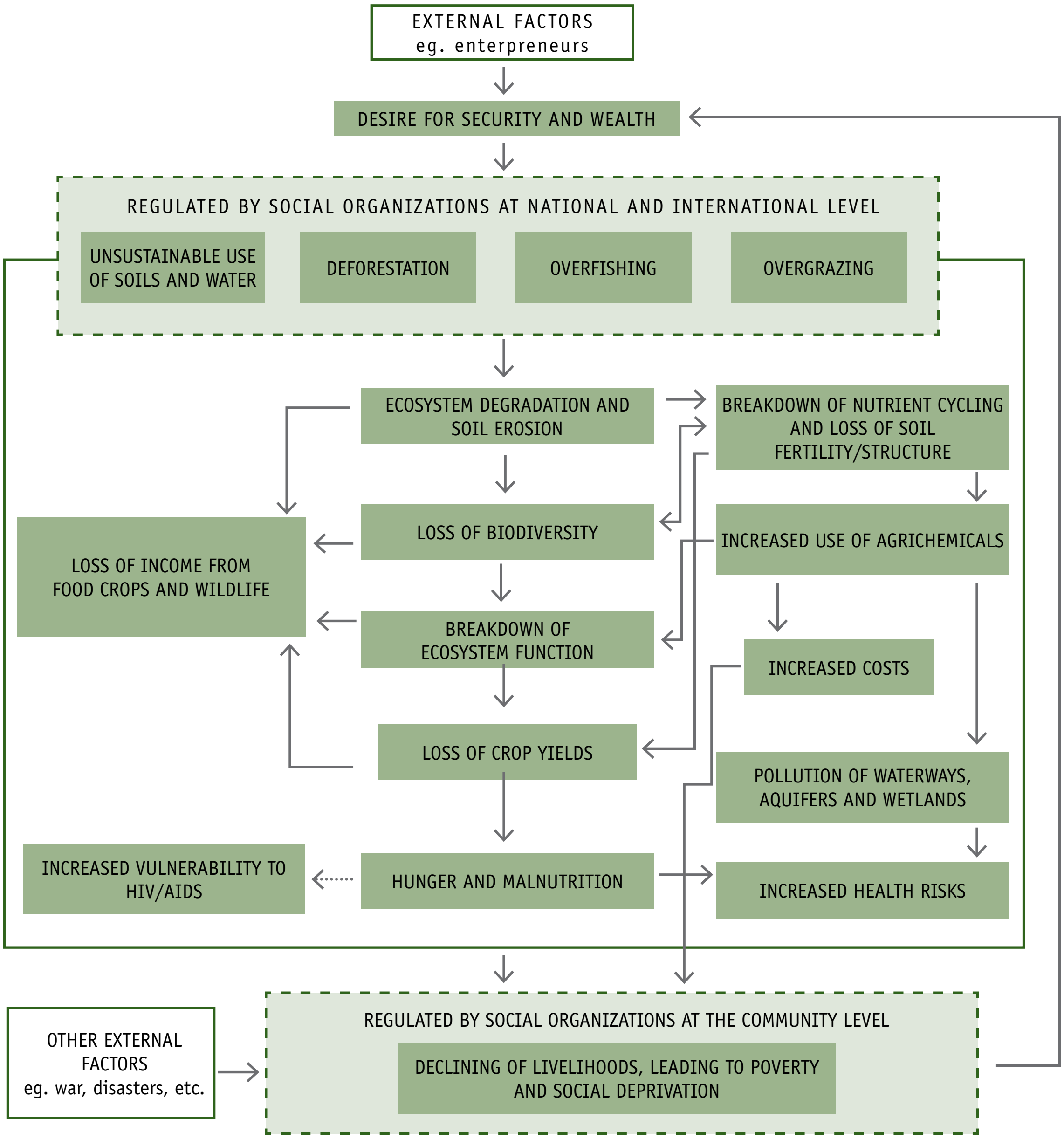
However, these benefits have come with a high environmental cost and only marginal improvements in reduced poverty, malnutrition and hunger in developing countries. Some of the major issues affecting global agriculture are:

- The scale of natural resource degradation (affecting 2.6 billion people and 2 billion ha of farm land), the depletion of soil fertility (nitrogen, phosphorus, and potassium deficiencies affecting 59, 85 and 90 percent of crop land, respectively), loss of biodiversity (valued at US$1 542 billion/yr), depletion of water resources (2 664 km^3/yr) and agro-ecosystem function, against a background in which new land for agriculture is increasingly scarce. This situation, which has arisen from the over-exploitation of natural capital, makes the rehabilitation of farm land, and its associated natural assets, an imperative.
- The incidence of poverty (3.2 billion people with an income of less than US$2/day), malnutrition, and nutrient deficiency (2 billion people) and hunger (0.9 billion people) remain at unacceptable levels, despite the very significant improvements in agricultural production. In addition, 1 billion people are affected by obesity due to poor diet.
- There are numerous organizational and conceptual “disconnects” between agricultural disciplines and organizations, especially those responsible for environmental services and sustainable development. Agricultural production and governance have focused on producing individual agricultural commodities rather than seeking synergies and the optimum use of limited resources through technologies promoting integrated natural resources management and multifunctional agriculture.
- Modern public-funded agricultural knowledge, science, and technology research and development has largely ignored the improvement of traditional production systems based on “wild” resources which, traditionally, have played an important role in peoples’ livelihoods.
- Agriculture is responsible for 15 percent of greenhouse gas emissions.
- Since the mid-20th Century, the Globalization pathway has dominated agricultural research and development as well as international trade, at the expense of the “Localization” benefits of many existing small-scale activities of farmers and traders that are aimed at meeting the needs of poor people at the community level.

Together, these issues contribute to the formation of a downward cycle of land degradation and associated social deprivation (Figure 2) that drive down crop yields and suppress farmers’ livelihoods, which together are responsible for a Yield Gap (Figure 3) between the biological potential of modern crop varieties and the yield that poor farmers typically manage to produce in the field (Leakey, 2010, 2012a).

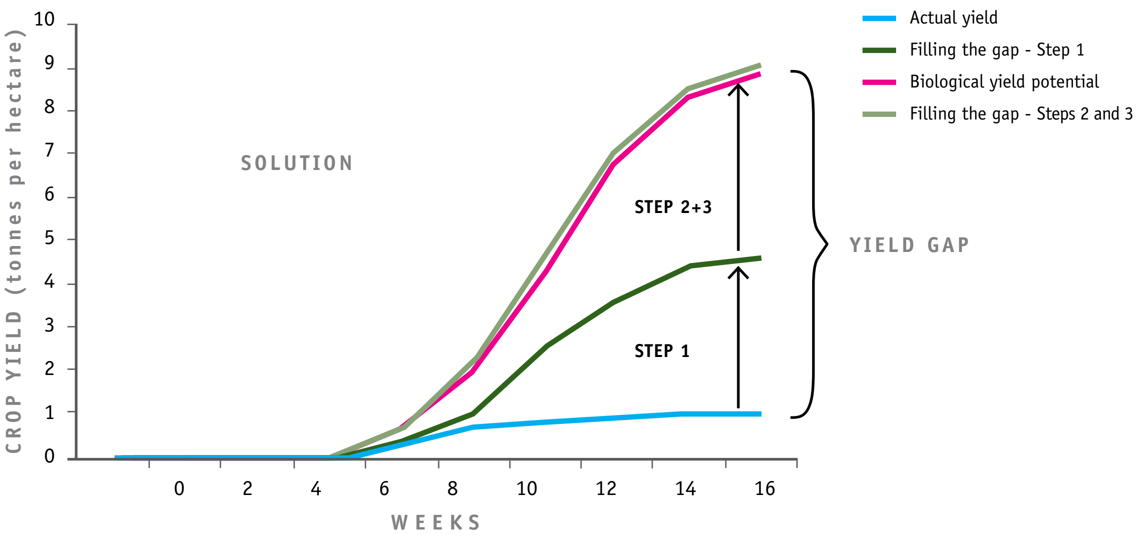
An analysis of the cycle of land degradation and associated social deprivation recognizes that the cycle is driven by a desire for security and wealth, which in turn drives deforestation, overgrazing and unsustainable use of soils and water: all of which cause agro-ecosystem degradation (Leakey, 2010, 2012a). In farmers’ fields this is seen as soil erosion, breakdown of nutrient cycling and the loss of soil fertility and structure. The consequence of this degradation is the loss of biodiversity, the breakdown of ecosystem functions and the loss of crop yield. Low crop yields result in hunger, malnutrition, increased health risks and a loss of income, all of which are manifest as declining livelihoods and so return the cycle to a desire for security and wealth. It is recognized that at all of the steps within this conceptual diagram, there are a range of socio-economic and biophysical influences which will determine the speed of the downward progress at any particular site. Such factors include: access to markets, land tenure and local governance - not to mention external factors such as natural disasters, conflict and war, and economic drivers such as international policy and trade agreements.

FIGURE 2. DIAGRAMMATIC REPRESENTATION OF THE CYCLE OF LAND DEGRADATION AND ASSOCIATED SOCIAL DEPRIVATION



Source: Leakey, 2012a.

FIGURE 3. DIAGRAMMATIC REPRESENTATION OF THE YIELD GAP AND THE STEPS REQUIRED TO CLOSE THE GAP



Source: Leakey, 2012a.

PRINCIPLE 10. Rehabilitate degraded land and reverse social deprivation: Close the Yield Gap.

To be productive, conventional approaches to modern agriculture typically require large inputs of fertilizers, pesticides, mechanization and, in dry areas, irrigation. However, the dependence of this type of agriculture on income and financial capital makes it inaccessible to hundreds of millions of poor farmers due to their high cost and local availability. As it is clear that cutting more forest down for agriculture is not an acceptable option, it is crucial to find ways of making degraded land productive again. Unfortunately, agricultural research and development has focused more on increasing potential yield than on addressed the cycle of land degradation and social deprivation that creates the Yield Gap.

To close the Yield Gap, Leakey (2010, 2012a) has suggested the following three-step approach as a way forward, using example of maize (*Zea mays L.*) production in eastern and southern Africa. The approach is based on the use of agroforestry fallows, perennial crops, tree domestication, and the marketing of agroforestry tree products as a way deliver multifunctional agriculture:-

- **Step 1.** Adopt agroforestry technologies such as two year improved fallows or relay cropping with nitrogen-fixing shrubs that improve food security by raising maize yields four-fold from around 1 Mg ha-1 (Buresh and Cooper, 1999; Sileshi *et al.* 2008). Likewise, stands of *Faidherbia albida* (Del.) A. Chev. trees play a similar role in the so-called Evergreen Agriculture (Garrity, 2012; Swaminathan, 2012). This allows the farmers to reduce the area of their holdings planted with maize and so make space for other crops, perhaps cash crops which would generate income. This diversification could also include the establishment of perennial grains. An additional benefit arising from improved fallows with leguminous shrubs like *Sesbania sesban* (L.) Merr. and *Desmodium* spp. is the reduction of parasitic weeds like *Striga hermonteca Benth*., and the reduced incidence of insects pests like the stem borers of maize (Cook *et al.* 2007).

- **Step 2.** Adopt the Participatory Domestication of indigenous trees producing marketable products, so that new, locally important and nutrient-rich cash crops are rapidly developed as a source of income and products of day-to-day domestic importance, and help empower women and maintain culture and traditions (Cooper *et al.* 1996; Sanchez and Leakey, 1997). Sale of these products would allow the purchase of fertilizers and so, potentially, the increase of maize yields up to 10 Mg ha-1. Consequently, the area under maize could be reduced further to allow more cash cropping. Filling the Yield Gap will also maximize returns on past investments in food crop breeding.
- **Step 3.** Promote entrepreneurism and develop value-adding and processing technologies for the new tree crop products, so increasing availability of the products throughout the year, expanding trade and creating employment opportunities – outputs which should help to reduce the incidence of poverty.

This approach, which is based on good land husbandry to rebuild natural soil fertility and health, therefore increases food security by improving crop yields. However, it does more than that. The inclusion of trees and other perennial crops within farming systems increases the number of niches in the agro-ecosystem. These are filled by a wide range of organisms (the unplanned biodiversity) in ways that improve nutrient, carbon and hydrological cycles; enrich food chains and meet the needs of more complex food cycles, and reduce the risks of pest and disease outbreaks. As the trees increase in size and the ecosystem progresses towards maturity, the numbers of niches for further ecosystem diversity continues to increase further enhancing agro-ecosystem function and services. This diversification makes these farming systems less damaging and more sustainable. The high species diversity of moist and dry tropical forests and woodlands means that there are many species available to play these important ecological roles in a developing agro-ecological succession (Leakey, 1996). The domestication of indigenous trees as new crop plants offer opportunities to increase the numbers of cultivated plants (the ‘planned biodiversity’) in these systems in ways that increase the wild organisms (the ‘unplanned biodiversity’) that fills the niches in the diversified farming system. The new crops of course also provide products to meet the social and economic needs of poor farmers (70 percent of the 3.2 billion people living on less than US$2 per day) for food self-sufficiency, micronutrients, medicines and all their other day-to-day needs not provided by modern monocultures. An important part of this approach is therefore to ‘hedge’ against environmental and ecological risk and provide the livelihood needs of the local communities.

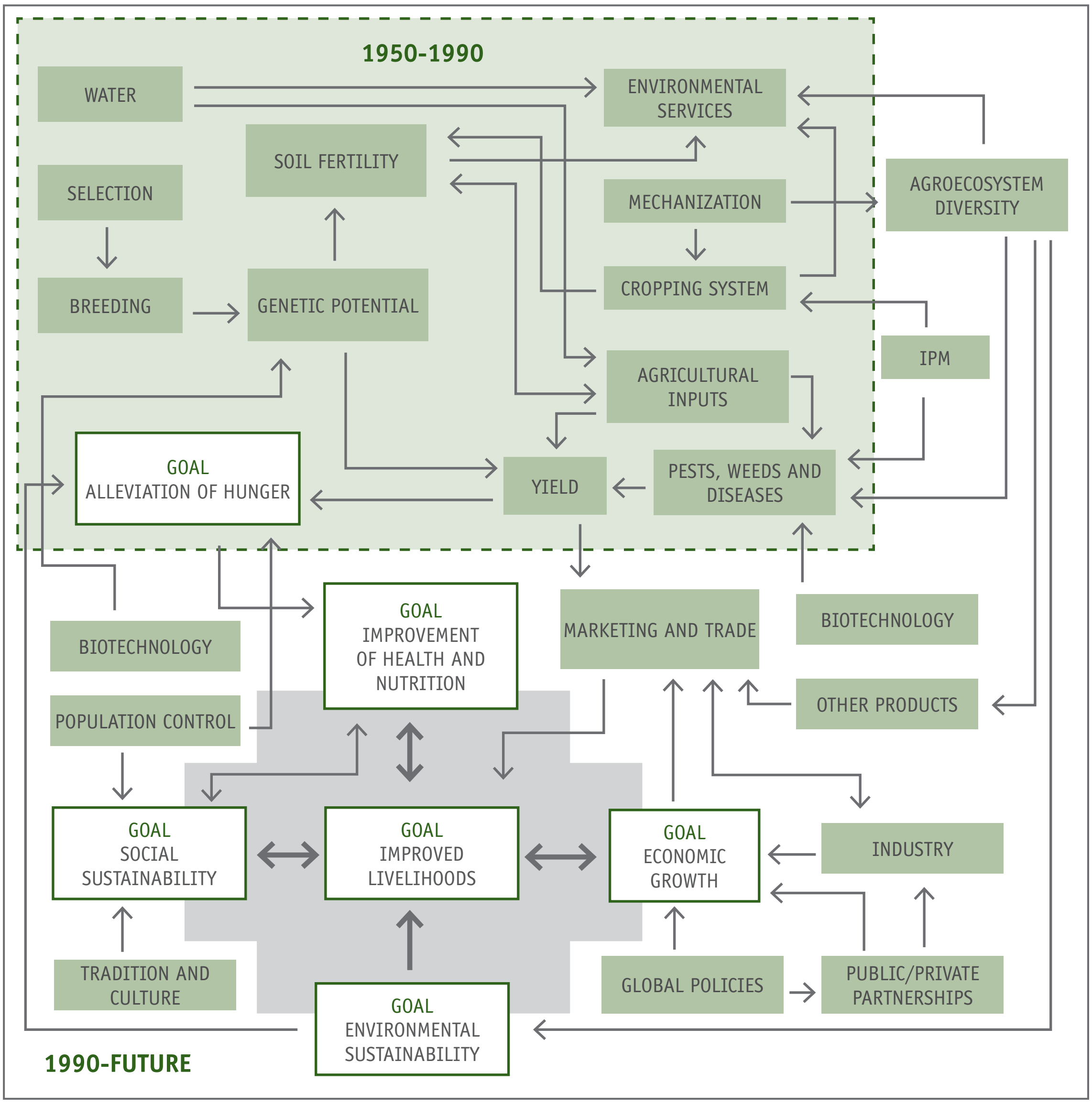
By including the domestication of traditional food species and the marketing of their products, this approach also meets the needs of the community for micronutrients that mitigate malnutrition and boost immunity to diseases (Leakey *et al.* 2012; Leakey, 2012a/b). Concomitantly, the commercialization of the tree products matches the product value chain to the needs of traders for more uniform and higher quality products with improved shelf life. This emphasis on enhanced trade is then being found to open up a pathway out of poverty based on new sources of employment and new local business opportunities (Leakey 2012a). So, as a package, this combination of social- and economic advancement with the environmental restoration creates a generic model for closing the Yield Gap – a model which is highly adaptable to a very wide range of climatic and edaphic environments and to numerous socio-economic situations, on account of the very large numbers of candidate tree species appropriate to all environments (Leakey, 2010; Leakey, 2012a,b).

PRINCIPLE 11. Promote 'Multi-functional Agriculture' for environmental/social/economic sustainability and relief of hunger, malnutrition, poverty and climate change.

Multifunctional agriculture, as described by International Assessment of Agricultural Science and Technology for Development (IAASTD) (McIntyre *et al.* 2008), has the objective of simultaneously promoting the social, economic and environmental benefits of farming systems. In other words, agriculture is very much more than just the production of food (Figure 4).

Agroforestry is particularly relevant to the delivery of multi-functional agriculture as it addresses: (i) environmental issues: (a) soil fertility management, (b) the rehabilitation of degraded farming systems, (c) loss of biodiversity above and below ground, (d) soil and watershed protection, (e) carbon sequestration and (f) energy needs through the provision of wood fuel; (ii) Economic issues: (a) income generation through trade in useful and marketable tree products, (b) the creation of business and employment opportunities in trade and value-adding through the processing of tree and non-tree products and (c) the creation of new cottage industries for diversification and enrichment of the rural economy; (iii) Social issues: (a) lack of gender equity and the need for community empowerment, (b) urban migration, (c) poverty and health related problems, (d) loss of cultural identity and of Traditional Knowledge, (e) loss of food sovereignty, (f) the lack of income for better education and training, provision of essential skills, and (g) the lack of income for community projects such as the supply of potable water, community infrastructure developments, transport, etc.

FIGURE 4. DIAGRAMMATIC REPRESENTATION OF MULTIFUNCTIONAL AGRICULTURE AND ITS GOALS.



Together, the above benefits help to resolve the higher level livelihood issues of: (i) a lack of food and nutritional security - and associated poor health, (ii) extreme and widespread poverty, (iii) the loss of self-esteem arising from the marginalization of poor communities by the social elite and the consequent vulnerability to exploitation arising from a lack of self-sufficiency, (iv) deforestation and over-exploitation of natural resources, (v) the lack of available productive land due to the degradation of complex mature and functioning agro-ecosystems and the fragmentation of agricultural landscapes (Perfecto and Vandermeer, 2010; Leakey, 2010; van Noordwijk *et al.* 2012).

With the increasing recognition of the need to address climate change the integration of trees in farming systems is being recognized as crucial for the reduction of greenhouse gas emissions and climate smart agriculture (Nair, 2012; van Noordwijk *et al.* 2011). Large perennial trees have a high volume of standing biomass and through litter fall and root turnover they also enrich the soil with carbon (Minang *et al.* 2012). Studies suggest that the conversion of degraded farm land to mature agroforest could increase carbon per hectare from 2.2 to 150 mg over a potential area of 900 million ha worldwide (World Agroforestry Centre, 2007).

So, we see that by using agroforestry to resolve the production, food and nutritional security and poverty issues causing the Yield Gap we simultaneously move farming systems towards the objectives of multifunctional agriculture and create an approach to tropical agriculture which both builds on the positive outcomes of the last 60 years of the Green Revolution, and addresses some of its negative outcomes. As a consequence, tropical agriculture becomes more productive – a process of intensification - yet environmentally, socially and economically more sustainable that the current conventional approach to modern agriculture (Leakey, 2012c).

PRINCIPLE 12. **Encourage Integrated Rural Development.**

So far, we have seen that agroforestry has two important roles in the development process relating to agriculture and the rural economy: i) it provides techniques for the implementation of a highly adaptable set of three steps for the closure of the Yield Gap that includes value-adding within the marketing of a wide range of indigenous tree products from mixed farming systems, and ii) it is a delivery mechanism for intensified multifunctional agriculture. While these are big steps towards more sustainable rural development, they need to be set within an even wider context in which agroforestry and multifunctional agriculture are part of a regional programme of integrated rural development.

To pull the above 11 principles together into a single project, the World Agroforestry Centre in Cameroon initiated a development programme in 1998 centred around the provision of training in agroforestry for the rehabilitation of degraded land and the domestication/commercialization of fruits and nuts from indigenous trees. This was implemented in a participatory manner through Rural Resource Centres which in addition provided training in nursery management, entrepreneurism and the use of microfinance, community organization and infrastructure development, fabrication of simple tools and equipment for value-adding tree and non-tree food products and the expansion of the value chain for traditional food products.

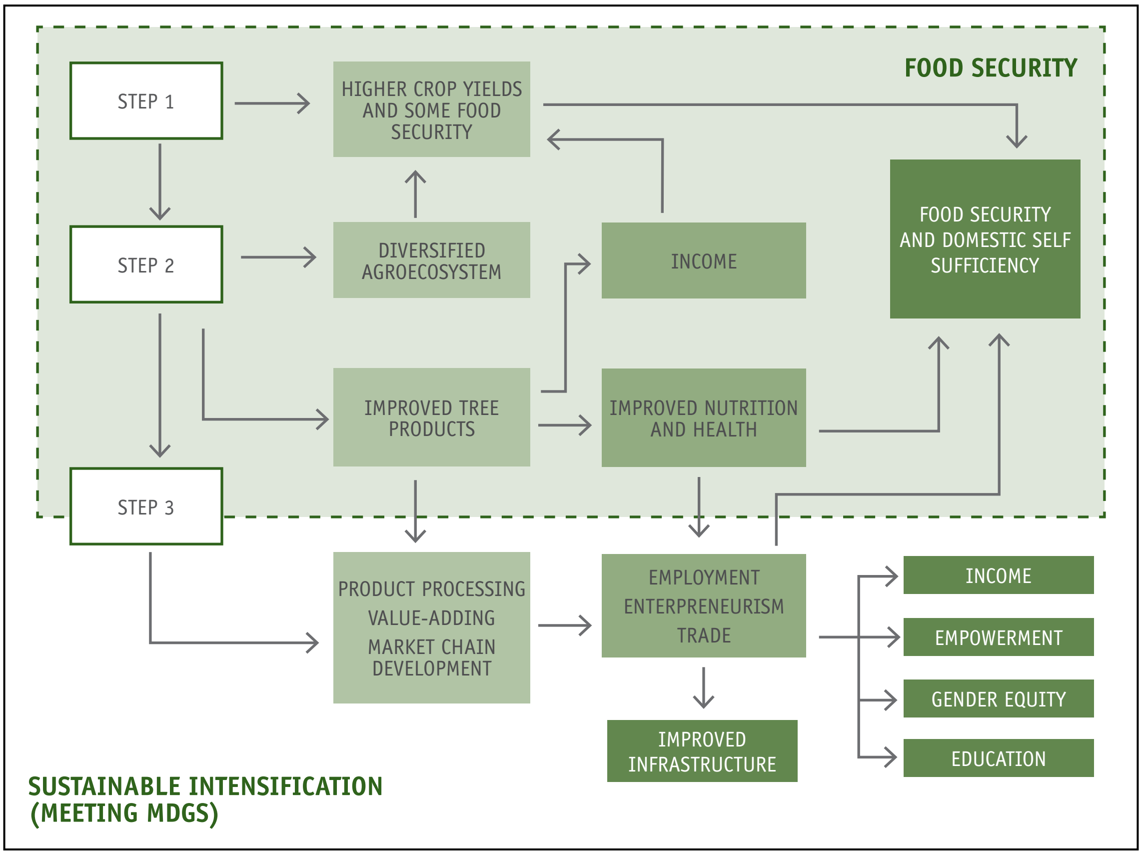
In this longest-running example of participatory domestication in agroforestry trees the researchers fed their outputs to NGO partners through training-of-trainers courses and by acting as mentors to the NGO-managed Rural Resource Centres established in pilot villages (Tchoundjeu *et al.* 2002, 2006, 2010; Asaah *et al.* 2011). The farmers in this partnership contributed their knowledge about the use and importance of local species, the range of variation in different traits of relevance to genetic selection and their Traditional Knowledge about the role of these species in local culture and tradition. They have also contributed their time and labour. Furthermore and crucially, they also made available some of their trees for research and for training in domestication techniques.

This case study - a winner of the prestigious Equator Prize – now involves more than 10 000 farmers and over 200 communities in the West and North-west regions of Cameroon, as well as entrepreneurs in local towns. The project is centred on five Rural Resource Centres which are providing a wide range of training to farmers through the growth of more than 120 satellite tree nurseries in surrounding communities supported by Relay Organizations (NGOs, CBOs, etc.) in the villages. The experience of the last 15 years indicates that the first income stream from agroforestry projects is derived from the sales of plants from village nurseries to neighbouring communities; and especially the sale of seedlings of nitrogen-fixing or the so-called ‘fertilizer’ trees (Asaah *et al.* 2011; Leakey and Asaah, 2013). In terms of soil fertility replenishment, the benefit flows from these trees are obtained relatively quickly (crop yield up two to three-fold in 2-3 years). On the other hand, it generally takes longer (>4 years) to obtain returns from the production and sale of the tree products. On average, results to date indicate that farmers’ income from the sale of plants from village nurseries has risen dramatically as the project gathers momentum (US$145, US$16 000 and US$28 350 after 2, 5, and 10 years, respectively).

In addition, to overcome one of the constraints to better food processing local metal workers in nearby towns have been supported to develop appropriate equipment for drying, chopping, and grinding a range of foodstuffs, including tree products not previously processed. The tree products are selling at higher than usual prices and in a few cases are being sent abroad. This component of the programme has created employment for metal workers and allowed local entrepreneurs to extend the shelf life and the quality of the produce they sell in local markets. For example, the fabrication of about 150 discharge mills and 50 dryers has generated income in excess of US$120 000 (Asaah *et al.* 2011; Leakey and Asaah, 2013). In parallel, women in nearby towns have set up businesses for grinding crops like cassava (*Manihot esculenta*) have also increased their income substantially. The largest of these groups was run by ten women who employed eight workers and processed about sixty-six 180kg-bags of dried cassava flour per day throughout the year. Profits from bags selling at US$40-US$54 per bag, depending on the season, were said to be more than US$2.5 per bag. When integrated with developments across in the agricultural sector, small business developments such as these benefit from linkages with microfinance, business training and better access to simple equipment for the processing and packaging of raw products.

From the above it is clear that the commercialization of sustainably grown products delivers really important impacts from agroforestry and multifunctional agriculture (Figure 5). However, we have to recognize that commercialization that can also pose great risks affecting the success or failure of the overall initiative. One study has found that bottom-up community initiatives like those described here have the greatest chance of being 'winners', although if the companies involved recognize the importance of buying raw products from local smallholder producers, top-down commercialization can also be effective (Wynberg *et al.* 2003).

FIGURE 5. DIAGRAMMATIC REPRESENTATION OF HOW THE THREE STEPS TO CLOSE THE YIELD GAP IMPACT ON FOOD SECURITY, POVERTY AND LIVELIHOODS (SUSTAINABLE INTENSIFICATION)



One important and exciting thing about the Cameroon project has been the wide range of positive livelihood impacts that the farmers are saying have truly transformed their lives (Leakey and Asaah, 2013). These require further quantification and verification, but include: substantially increased income, new employment opportunities, improved nutrition, improved health from potable water and better diets, and the ability to spend money on children's schooling, home improvements, wells, etc. Significantly, one of the outcomes mentioned by young people in the participating communities is that this now means that they can see a future for themselves if they remain in the village rather than feeling that they have to migrate to towns and cities for a better life. In addition, women have indicated that improved infrastructure (wells, roads, etc.) has reduced the drudgery in their lives as a result of not having to collect water from rivers and carry farm produce from remote farms. These benefits, like the mechanical processing of food crops, have meant that they had more time to look after their families and engage in farming or other income generating activities.

It is encouraging that the levels of income generation achieved in Cameroon, albeit on a very small scale, exceed those proposed in the Millennium Development Goals. This and the other impacts presented here strongly suggest that by promoting self-sufficiency through the empowerment of individuals and community groups through the provision of new skills in agroforestry, tree domestication, food production and processing, community development, and microfinance, it is possible for communities to climb the entrepreneurial ladder out of poverty, malnutrition, and hunger. What is needed now is to disseminate this approach to millions of other poor people in Africa and other tropical countries.

To conclude, through the integration of rural development activities, farmers in Cameroon are intensifying their farming systems in ways that are environmentally, socially and economically more sustainable, while people in local villages and small towns are developing cottage industries and engaging more in marketing and trade. The consequence of this has been the start of the climb out of poverty and entry into the cash economy. This relationship between enhanced farm production and urban life is important for the rural economy as it is an example of farm production being the 'engine of growth'. This is perhaps the start of a new approach to rural development in the tropics – one that perhaps replicates what happened thousands of years ago in the Near East and Europe as cereals and other staple food crops were domesticated and brought into cultivation. Interestingly, Diamond (1997) has credited the domestication of food crops with the advance of western civilization. Recognizing this power of crop domestication, Leakey (2012a/d) has called for a 'new wave of domestication' to benefit people in developing countries who did not greatly benefit from the first wave. In this regard, one interesting development in recent years has been the involvement of a few multinational companies in Public-Private Partnerships with rural communities engaged in production of agroforestry products in tropical countries (Jamnadass *et al.* 2011; Leakey, 2012a). Although associated with risks, this also offers great opportunities for the future development of agroforestry tree crops if the strategies and practices can be developed appropriately.

SUSTAINABLE INTENSIFICATION

Currently, there is great interest internationally in seeking 'sustainable intensification' (Garnett and Godfray, 2012; Garnett *et al.* 2013). This paper presenting 12 principles for achieving both better and more food from mature perennial agro-ecosystems seeks to contribute to this debate and illustrate how the domestication of indigenous trees producing high value products, such as traditional foods and medicines, can be a catalyst for sustainable and integrated rural development. This paper also emphasises that an important strategy within this approach to sustainable intensification is the implementation of steps to restore productivity to degraded land and close the Yield Gap and meet the needs of a growing human population without the need for further deforestation (Figure 5; Leakey, 2012a). Clearly, the challenge for the future is to scale up the application of the principles outlined here to have meaningful impact on national, regional and global scales. A key to achieving this will be the attainment of political will. Towards this end, the IAASTD (McIntyre *et al.* 2009) placed a need for greater emphasis on:-

- Integrated approaches to land use management involving participatory approaches to planning and implementation
- Less exploitative approach to natural resources, especially soils and water, and a lower dependence on inorganic inputs and fossil energy
- Good husbandry to support agro-ecosystem health, restoration of degraded land and the reduction of the 'Yield Gap'.
- Increased involvement of local user groups in actions to improve natural resources management.
- Diversification of agriculture for improved soil amelioration, pest and disease control, and new marketable products.
- The domestication of new nutritious and marketable crops from local species, especially trees, to diversify diets and the local economy.
- Enhancement of rural livelihoods by meeting the needs of local people and supporting culture and tradition.
- Better integration of agricultural sectors, government departments and institutions, communities, and stakeholders to overcome "disconnects" in policy and practice.
- Public–private partnerships involving diverse stakeholder groups at the local level to support sustainable production, and in-country processing and value-adding.
- There is strong accord between these pointers to a better future for agriculture from IAASTD and the principles outlined in this paper.

REFERENCES

Abbiw, D. 1990. *Useful plants of Ghana*. Intermediate Technology Publications, London. 337pp.

Asaah, E.K., Tchoundjeu, Z., Leakey, R.R.B., Takousting, B., Njong, J. & Edang, I. 2011. Trees, agroforestry and multifunctional agriculture in Cameroon. *International Journal of Agricultural Sustainability*. 9: 110-119.

Buresh, R.J. & Cooper, P.J.M. 1999. The science and practice of short-term fallows. *Agroforestry Systems*. 47: 1-358.

Cook, S.M., Khan, Z.R. & Pickett, J.A. 2007. The use of 'push-pull' strategies in integrated pest management. *Annual Review of Entomology*. 52: 375-400.

Cooper, P.J.M., Leakey, R.R.B., Rao, M.R. & Reynolds, L. 1996. Agroforestry and the mitigation of land degradation in the humid and sub-humid tropics of Africa. *Experimental Agriculture*. 32: 235-290.

Cosyns, H., Degrande, A., de Wulf, R., van Damme, P. and Tchoundjeu, Z. 2011. Can commercialization of NTFPs alleviate poverty? A case study of Ricinodendron heudelottii (Baill.) Pierre ex Pax. kernel marketing in Cameroon. *Journal of Agriculture and Rural Development in the Tropics and Subtropics*. 113: 45–56.

Cunningham, A.B. 2001. *Applied Ethnobotany: People, Wild Plant Use and Conservation*. Earthscan, London, 300pp.

Degrande, A., Schreckenberg, K., Mbosso, C., Anegbeh, P.O., Okafor, J. & Kanmegne, J. 2006. Farmers' fruit tree growing strategies in the humid forest zone of Cameroon and Nigeria. *Agroforest Systems*. 67: 159-175

Degrande, A., Franzel, S., Yeptiep, Y.S., Asaah, E., Tsobeng, A. & Tchoundjeu, Z. 2012. Effectiveness of grassroots organisations in the dissemination of agroforestry innovations. In: *Agroforestry for Biodiversity and Ecosystem Services – Science and Practice*, Elsevier, 141-164

Diamond, J. 1997. *Guns, Germs and Steel: The Fates of Human Societies*. W.W. Norton & Co, London, UK.

Foundjem-Tita, D., Tchoundjeu, Z., Speelman, S., D'Haese, M., Degrande, A., Asaah, E., van Huylenbroeck, G., van Damme, P. & Ndoye, O. 2012 Policy and legal frameworks governing trees: Incentives or disincentives for smallholder tree planting decisions in Cameroon? *Small-scale Forestry*. DOI 10.1007/s11842-012-9225-z.

Franzel, S., Jaenicke, H. & Janssen, W. 1996. Choosing the right trees: setting priorities for multipurpose tree improvement. *ISNAR Research Report*. 8: 87 p.

Franzel, S., Akinnifesi, F.K. & Ham, C. 2008. Setting priorities among indigenous fruit tree species in Africa: examples from southern, eastern and western Africa regions. In: *Indigenous Fruit Trees in the Tropics: Domestication, Utilization and Commercialization*. Akinnifesi FK, Leakey RRB, Ajayi OC, Sileshi G, Tchoundjeu Z, Matakala P, Kwesiga FR (eds), CABI, Wallingford, UK, 1-27.

Garnett, T. & Godfray, H.C.J. 2012. Sustainable intensification in agriculture: Navigating a course through competing food system priorities. *Food Climate Research Network and the Oxford Martin Programme on the Future of Food*. University of Oxford, UK.

Garnett, T., Appleby, M.C., Balmford, A., Bateman, I.J., Benton, T.G., Bloomer, P., Burlingame, B., Dawkins, M., Dolan, L., Fraser, D., Herrero, M., Hoffmann, I., Smith, P., Thornton, P.K., Toulmin, C., Vermeulen, S.J. & Godfray, H.C.J. 2013. Sustainable intensification in agriculture: Premises and policies. *Science*. 341: 33-34.

Garrity, D. 2012. Agroforestry and the future of global land use. In: Agroforestry – The Future of Global Land Use, Nair PKR and Garrity, D (eds.). *Advances in Agroforestry*. 9: 21-27. Springer, Dordrecht, Germany.

Gyau, A., Takoutsing, B., Degrande, A. & Franzel, S. 2012. Producers' motivation for collective action for kola production and marketing in Cameroon. *Journal of Agriculture and Rural Development in the Tropics and Subtropics*. 113: 43–50

ICRAF. 2012. *Agroforestry Tree Genetic Resources Strategy 2013-2017*, World Agroforestry Centre PO Box 30677-00100 Nairobi, Kenya, 19pp.

Jamnadass, R., Lowe, A., & Dawson, I.K. 2009. Molecular markers and the management of tropical trees: the case of indigenous fruits. *Tropical Plant Biology*. 2: 1-12.

Jamnadass, R., Dawson, I.K., Anegbeh, P., Asaah, E., Atangana, A., Cordeiro, N.J., Hendrick, H., Henneh, S., Kadu, C.A.C., Kattah, C., Misbah, M., Muchugi, A., Munjuga, M., Mwaura. L., Ndangalasi, H.J., Sirito Njau, C., Nyame, S.K., Ofori, D., Peprah, T., Russell, J., Rutatina, F., Sawe, C., Schmidt, L., Tchoundjeu, Z. & Simons, A.J. 2010. Allanblackia, a new tree crop in Africa for the global food industry: market development, smallholder cultivation and biodiversity management. *Forests, Trees and Livelihoods*. 19: 251-268.

Kiptot, E. & Franzel, S. 2012. Gender and agroforestry in Africa: who benefits? The African Perspective. In: Agroforestry – The Future of Global Land Use, Nair PKR and Garrity, D (eds.), *Advances in Agroforestry*. 9: 463-496. Springer, Dordrecht, Germany.

Leakey, R.R.B. 1996. Definition of agroforestry revisited. *Agroforestry Today*. 8(1): 5-7.

Leakey, R.R.B. 1999a. Potential for novel food products from agroforestry trees. *Food Chemistry*. 64: 1-14

Leakey, R.R.B. 1999b. Agroforestry for biodiversity in farming systems. In: *Biodiversity in Agroecosystems*. Collins, WW and Qualset CO (eds.), CRC Press, New York, p127-145.

Leakey, R.R.B. 2004. Physiology of vegetative reproduction. In: *Encyclopaedia of Forest Sciences*. (Burley J, Evans J, Youngquist JA (eds.), Academic Press, London, UK, 1655-1668.

Leakey, R.R.B. 2010. Agroforestry: a delivery mechanism for Multi-functional Agriculture. In: *Handbook on Agroforestry: Management Practices and Environmental Impact*, Kellimore LR (ed.), Nova Science Publishers. Environmental Science, Engineering and Technology Series, New York, USA, 461-471.

Leakey R.R.B. 2012a. *Living with the Trees of Life – Towards the Transformation of tropical Agriculture*, CABI, Wallingford, UK. 200pp.

Leakey, R.R.B. 2012b. Addressing the causes of land degradation, food / nutritional insecurity and poverty: a new approach to agricultural intensification in the tropics and sub-tropics. In: *Wake Up Before it is too Late: Make Agriculture Truly Sustainable Now for Food Security in a Changing Climate*. 192-198, UNCTAD Trade and Environment Review 2013, U. Hoffman (ed.), UN Publications, Geneva, Switzerland.

Leakey, R.R.B. 2012c. The intensification of agroforestry by tree domestication for enhanced social and economic impact. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*. 7: No: 035, 1-3.

Leakey, R.R.B. 2012d Participatory domestication of indigenous fruit and nut trees: New crops for sustainable agriculture in developing countries. In: *Biodiversity in Agriculture: Domestication, Evolution and Sustainability*. Gepts PL, Famula TR, Bettinger RL, Brush SB, Damania AB, McGuire PE, and Qualset CO (eds.) Cambridge University Press, Cambridge, UK, 479-501.

Leakey, R.R.B. In press a. Agroforestry: The participatory domestication of trees. In: *Encyclopedia of Agriculture and Food Systems*. van Alfen, N. *et al.* (eds.). Elsevier Publishers, UK.

Leakey, R.R.B. In press b. Cloning: Macro-propagation. In: *Encyclopedia of Agriculture and Food Systems*. van Alfen. N. *et al.* (eds.). Elsevier Publishers, UK.

Leakey, R.R.B. & Newton, A.C. (eds.). 1994. *Tropical Trees: Potential for Domestication, Rebuilding Forest Resources*, HMSO, London. 284 pp.

Leakey, R.R.B. & Simons, A.J. 2000. When does vegetative propagation provide a viable alternative to propagation by seed in forestry and agroforestry in the tropics and sub-tropics? In: *Problem of Forestry in Tropical and Sub-tropical Countries - The Procurement of Forestry Seed - The Example of Kenya*. Wolf, H. & Arbrecht, J. (Eds), 67-81, Ulmer Verlag, Germany.

Leakey, R.R.B. & Page, T. 2006. The 'ideotype concept' and its application to the selection of 'AFTP' cultivars. *Forests, Trees and Livelihoods*. 16: 5-16.

Leakey, R.R.B. & Akinnifesi, F.K. 2008 Towards a domestication strategy for indigenous fruit trees in the tropics. In: *Indigenous Fruit Trees in the Tropics: Domestication, Utilization and Commercialization*. Akinnifesi, F.K., Leakey, R.R.B., Ajayi, O.C., Sileshi, G., Tchoundjeu, Z., Matakala, P. and Kwesiga, F. (eds), CAB International, Wallingford, UK, 28-49.

Leakey, R.R.B. & Asaah, E.K. 2013. Underutilised species as the backbone of multifunctional agriculture – The next wave of crop domestication. *Acta Horticulturae.* 979: 293-310.

Leakey, R.R.B. & van Damme, P. 2014. *The role of tree domestication in value chain development*.

Leakey, R.R.B., Mesén, J.F., Tchoundjeu, Z., Longman, K.A., Dick, J.McP., Newton, A.C., Matin, A., Grace. J., Munro, R.C. & Muthoka, P.N. 1990. Low-technology techniques for the vegetative propagation of tropical tress. *Commonwealth Forestry Review.* 69: 247-257.

Leakey, R.R.B., Schreckenberg, K. & Tchoundjeu, Z. 2003. The participatory domestication of West African indigenous fruits. *International Forestry Review.* 5: 338-347.

Leakey, R.R.B., Tchoundjeu, Z., Schreckenberg, K., Shackleton, S.E. & Shackleton, C.M. 2005. Agroforestry tree products (AFTPs): targeting poverty reduction and enhanced livelihoods. *International Journal in Agricultural Sustainability*. 3: 1-23

Leakey, R.R.B., Weber, J.C., Page, T., Cornelius, J.P., Akinnifesi, F.K., Roshetko, J.M., Tchoundjeu, Z. & Jamnadass, R. 2012. Tree domestication in agroforestry: progress in the second decade. In: *Agroforestry – The Future of Global Land Use*. 145-173, Nair, P.K., and Garrity, D. (eds.), Springer, USA.

Lombard, C. & Leakey, R.R.B. 2010. Protecting the rights of farmers and communities while securing long term market access for producers of non-timber forest products: experience in southern Africa. *Forests, Trees and Livelihoods*. 19: 235-249.

Mbosso, C., Degrande, A., Tabougue, P., Franzel, S., van Noordwijk, M., van Damme, P., Tchoundjeu, Z., & Foundjem-Tita, D. 2014. No appropriate technology so far for Ricinodendron heudelotii (Baill. Pierre ex Pax) nut cracking in Cameroon: Performance of a mechanized cracker. *African Journal of Agricultural Science*.

McIntyre BD, Herren H, Wakhungu, J. & Watson, R. (eds.). 2008. *International Assessment of Agricultural Knowledge Science and Technology for Development: Global Report*. Island Press, New York, USA, 590pp.

Minang, P.A., van Noordwijk, M. & Swallow, B. 2012. High-carbon-stock rural development pathways in Asia and Africa: How improved land management can contribute to economic development and climate change mitigation. In: Agroforestry – The Future of Global Land Use, Nair PKR and Garrity, D (eds.), *Advances in Agroforestry*. 9: 127 - 144. Springer, Dordrecht, Germany.

Nair, P.K.R. 2012. Climate change mitigation: A low-hanging fruit of agroforestry. In: Agroforestry – The future of global land use, Nair PKR and Garrity, D (eds.), *Advances in Agroforestry*. 9: 31-68. Springer, Dordrecht, Germany.

Perfecto, I. & Vandermeer, J. 2010. The agroecological matrix as alternative to the land sparing/ agricultural intensification model. *Proceedings of the National Academy of Sciences of the United States of America*. 107: 5786-5791.

Pauku, R.L., Lowe, A. & Leakey, R.R.B. 2010. Domestication of indigenous fruit and nut trees for agroforestry in the Solomon Islands. *Forests, Trees and Livelihoods*. 19: 269-287.

Sanchez, P.A. & Leakey, R.R.B. 1997. Land use transformation in Africa: three determinants for balancing food security with natural resource utilization. *European Journal of Agronomy*. 7: 15-23.

Schreckenberg, K., Awono, A., Degrande, A., Mbosso, C., Ndoye, O. & Tchoundjeu, Z. 2006a. Domesticating indigenous fruit trees as a contribution to poverty reduction. *Forests, Trees and Livelihoods*. 16: 35-51.

Schreckenberg, K., Marshall, E., Newton, A., Te Velde, D.W., Rushton, J. & Edouard, F. 2006b. *Commercialisation of non-timber forests products: what determines success*. ODI Forestry Briefing. Overseas Development Institute, London, UK.

Sileshi, G., Akinnifesi, F.K., Ajayi, O.C. & Place, F. 2008. Meta-analysis of maize yield response to planted fallow and green manure legumes in sub-Saharan Africa. *Plant and Soil.* 307: 1–19.

Simons, A.J. 1996. ICRAF's strategy for domestication of indigenous tree species. In: Domestication and Commercialization of Non-Timber Forest Products in Agroforestry Systems, Leakey, R.R.B., Temu, A.B., Melnyk, M. and Vantomme, P. (eds.), *Non-Wood Forest Products*. 9: 8-22, FAO Rome, Italy.

Simons, A.J. & Leakey, R.R.B. 2004. Tree domestication in tropical agroforestry. *Agroforestry Systems.* 61: 167-181.

Swaminathan, M.S. 2012. Agroforestry for an ever-green revolution. In: Agroforestry – The Future of Global Land Use, Nair PKR and Garrity, D (eds.), *Advances in Agroforestry*. 9: 7-10. Springer, Dordrecht, Germany.

Tchoundjeu, Z., Kengue J. & Leakey, R.R.B. 2002. Domestication of *Dacryodes edulis*: state-of-the art. *Forests, Trees and Livelihoods.* 12: 3-14.

Tchoundjeu, Z., Asaah, E., Anegbeh, P.O., Degrande, A., Mbile, P., Facheux, C., Tsobeng, A., Atangana, A.R. & Ngo-Mpeck, M-L. 2006. Putting participatory domestication into practice in West and Central Africa. *Forests, Trees and Livelihoods.* 16: 53-70.

Tchoundjeu, Z., Degrande, A., Leakey, R.R.B., Simons, A.J., Nimino, G., Kemajou, E., Asaah, E., Facheux, C., Mbile, P., Mbosso, C., Sado, T. & Tsobeng, A. 2010. Impact of participatory tree domestication on farmer livelihoods in west and central Africa. *Forests, Trees and Livelihoods.* 19: 219-234.

van Noordwijk, M., Hoang, M.H., Neufeldt, H., Öborn, I. & Yatich, T. (eds). 2011. How trees and people can co-adapt to climate change: reducing vulnerability through multifunctional agroforestry landscapes. *World Agroforestry Centre,* Nairobi, Kenya, 131pp.

van Noordwijk, M., Tata, H.L., Xu, J., Dewi, S. & Minang, P. 2012. Segregate or integrate for multifunctionality and sustained change through landscape agroforestry involving rubber in Indonesia and China In: Agroforestry – The Future of Global Land Use, Nair PKR and Garrity, D (eds.). *Advances in Agroforestry*. 9: 69-104. Springer, Dordrecht, Germany.

Wynberg, R.P., Laird, S.A., Shackleton, S., Mander, M., Shackleton, C., du Plessis, P., den Adel, S., Leakey, R.R.B., Botelle, A., Lombard, C., Sullivan, C., Cunningham, A.B. & O'Regan, D. 2003. Marula policy brief. Marula commercialisation for sustainable and equitable livelihoods. *Forests, Trees and Livelihoods*. 13: 203-215.

23

PERENNIAL CROPS AND TREES: TARGETING THE OPPORTUNITIES WITHIN A FARMING SYSTEMS CONTEXT

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INTRODUCTION: THE SEARCH FOR SUSTAINABILITY

Sustainable and resilient intensification of farming systems during the coming decades is a central challenge of our times. Almost half the world — over 3 billion people — live on less than US$2.50 per day; and approximately 1.3 billion, or about 22 percent of the population, consume less than US$1.25 per day (Chen and Ravallion, 2012). The immediate imperative is improving the household food security, incomes and livelihoods of the 1.3 billion poor: and the future challenge is to expand food production in order to feed the forecasted 9 billion consumers in 2050.

Economic growth is necessary to reduce poverty and food insecurity, but it is not sufficient (FAO, 2012). The majority of extremely poor households depend on agriculture for a significant part of their livelihoods, so it is not surprising that agricultural development is particularly effective in stimulating economic growth and reducing hunger and malnutrition (World Bank, 2008). Smallholder-based agricultural growth increases returns to labour and generates employment, especially for poor women. Dixon *et al.* (2001) identified five pathways by which farm households increase income and escape poverty: intensification (of existing patterns of production), diversification (sometimes bundled with intensification), expansion of operated farm size, increased off-farm income and exit from agriculture. Some recent improvement in household food security is reflected in the Global Hunger Index (IFPRI, 2013), but the progress is uneven and natural resources which underpin future agricultural productivity and food production are under increasing pressure.

Sustainability is a major concern given the pressure on land, water and energy resources (Lee and Barrett, 2001). Alongside the competition from other sectors for land, water and energy resources, the land frontier is approaching closure (Deininger and Byerlee, 2011). The pressure on land, water, energy and food is reflected in increasing prices during the recent past, notably increased resource valuations, the food price spike of 2008 and the forecasts of higher and more volatile food prices in coming decades.

The roadmap to achieve sustainable intensification is much debated (GO-Science, 2009; Tillman *et al.* 2011). The historic doubling of food production over the last four decades in Asia, largely due to the Green Revolution, was achieved through yield increases with limited additional land and water inputs, stemming from improved varieties complemented by improved fertilizer and crop management and functioning institutions and policies (Evenson and Gollin, 2003). The intensification of cropping systems through the Green Revolution was initially concentrated in well-watered areas with good connections to markets; and thus the initial livelihood benefits tended to be local whereas the food security dividends were regional or national. The Green Revolution was just one example of technology-driven changes which underpinned the growing intensification and differentiation of farming systems.

In relation to food production, recent analysis show significant variation of growth in total factor productivity across and within countries (Fuglie and Wang, 2012). Other analyses show growing concentrations of food production in a small number of annual commodities. For example, maize, wheat and rice production expanded relative to coarse grains and tubers; and chicken and pig production grew relative to cattle, sheep and goats (Tillman *et al.* 2011). However, even the growth in yield of the preferred cereal grains (roughly 40 kg/ha/y for wheat, 52 kg/ha/y for rice and 64 kg/ha/y for maize) lags behind growth in demand. Furthermore, there is evidence of some slowdown of annual cereal yield growth (Cassman, 2011). Moreover, in most food crop improvement programmes, the characteristic of perenniality has been neglected or removed through selection for yield. Over time, the role of perennials in food production has progressively diminished.

Quite apart from the core goals of global food security and economic growth, there are a spectrum of ecosystem services which are relevant to the search for inclusive sustainability – for example, biodiversity, water yield and purification and carbon sequestration (Asbjornsen *et al.* 2013). The authors are not aware of studies which compare the losses of annual and perennial species, but suspect that the former are more vulnerable to loss than the latter. Conversely, farmer re-vegetation initiatives show that the re-establishment of perennials is more challenging than for annuals. From the perspective of agro-ecosystem integrity and resilience, we argue that perennials tend to stabilize and enhance agro-ecosystem functions. This characteristic is critical for human outcomes, as the variability of productivity is a major source of the persistence of poverty, and also of 'new' poverty as households are stripped of assets and slide into poverty during (increasingly frequent) droughts. The important and well known ecosystem functions of perennials include increasing habitat niches for biodiversity (including crop pest predator habitats), reduced soil erosion and enhanced soil organic matter and moisture infiltration and storage, microclimate buffering, and greater above- and below-ground carbon sequestration.

As noted by other papers in this volume, perennial crops, pastures and trees potentially offer technical advantages for increased sustainable and resilient agricultural production (where agriculture is taken in the broad sense of crops, livestock, trees and fish). However, perennials must fit within farming systems which are shaped by agro-ecological and socio-economic factors. The following sections examine the added value from 'perenniality' (i.e., the functions potentially associated with perennial crops, pastures and trees) in eco-systems and farming systems, and identify a number of farming systems where perennials may have particular advantages. The adoption of perennials has implications for household livelihood improvement pathways. Because the future added-value of perennials depends very much on the evolution of farming systems, the main drivers of farming systems change are considered.

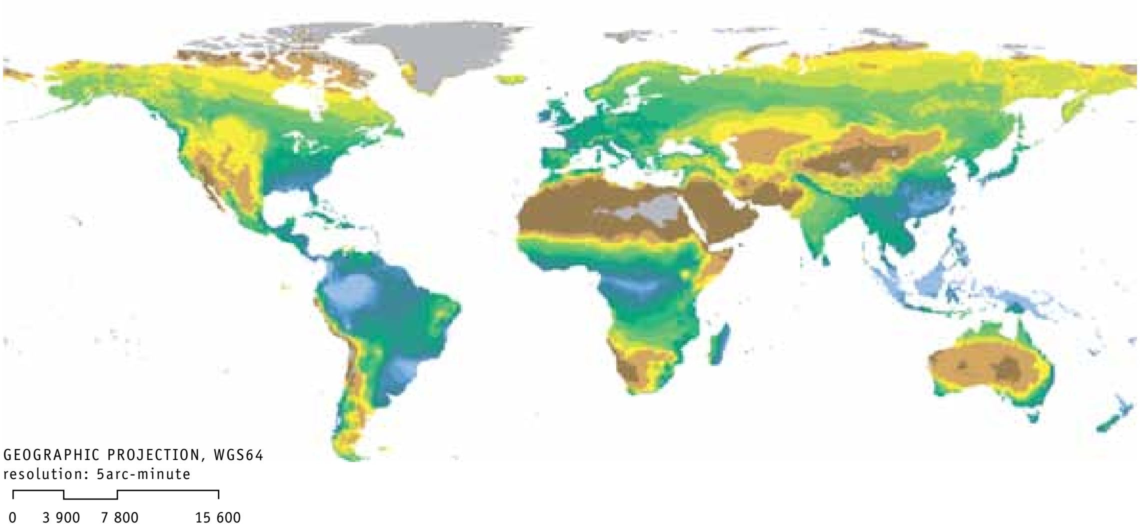
GEOGRAPHIC DISTRIBUTION OF FARMING SYSTEMS

Geography plays an important underlying role in the distribution of production and purchasing endowments. Except for irrigated areas, the average length of the growing period given precipitation, soils and temperatures is a major determinant of the potential productivity of rainfed crops, pastures and trees. Figure 1 illustrates the uneven distribution of lengh of the growing period across the surface of the globe. Moreover, the gaps between the achievable and actual yields of food crops are large in many environments especially in developing countries (Waddington *et al.* 2010).

FIGURE 1. GLOBAL DISTRIBUTION OF LENGTH OF GROWING PERIOD

GAEZ
Global Agro-Ecological Zones

REFERENCE LENGTH OF GROWING PERIOD ZONES (BASELINE 1961-1990)



DIMENSIONS

CROP	not applicable
WATER SUPPLY	not applicable
INPUT LEVEL	not applicable
YEAR	baseline period 1961-1990
SCENARIO	not applicable
CO2 FERTILIZATION	not applicable
VARIABLES	not applicable

LEGEND

⬜ 0.0: background
⬜ 1.0: 0 days
🟫 2.0: 1–29 days
🟫 3.0: 30–59 days
🟨 4.0: 60–89 days
🟩 5.0: 90–119 days
🟩 6.0: 120–149 days
🟩 7.0: 150–179 days
🟩 8.0: 180–209 days
🟩 9.0: 210–239 days
🟩 10.0: 240–269 days
🟦 11.0: 270–299 days
🟦 12.0: 300–329 days
🟦 13.0: 330–364 days
🟦 14.0: 0–365 -days
🟦 15.0: 0–365 days
🟦 16.0: 0–365 +days

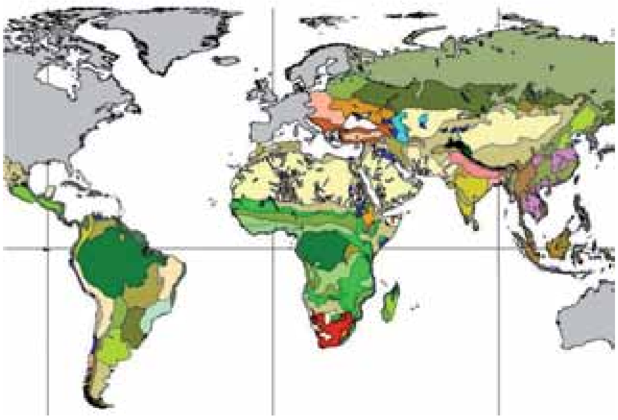
The various agro-ecologies are overlain by a mosaic of human settlement patterns that creates a multitude of diverse farming systems. Combining length of growing period and market access creates a pair of criteria which shape the land use, farming systems and livelihoods patterns of farmers in all countries. Following Dixon *et al.* (2001), a farming systems is defined as: ‘a population of individual farm systems that have broadly similar resource bases, enterprise patterns, household livelihoods and constraints, and for which similar development strategies and interventions would be appropriate. Depending on the scale of the analysis, a farming system can encompass a few dozen or many millions of households.’

At different scales, the concept would be applied in different ways – and heterogeneity would be apparent in different ways. Dixon *et al.* (2001) defined five dozen or so broad farming systems across six developing regions. Following the above concept, agro-ecology and socioeconomics shape crop, livestock and other farming system characteristics. Labour is an important household resource, and so off-farm employment is considered alongside crop and livestock production on the farm, and also domestic labour requirements, for example in the farm household. Each farming system has its own structure and function (Allan, 1965; Ruthenberg, 1971). There is remarkable diversity of farming systems in all regions of the world – ranging from productive banana-maize-coffee systems in the east African highlands to nomadic pastoralism of Central Asia to the maize soybean systems of the great plains of the United States. Figure 2 illustrates the five dozen most important farming systems across six developing regions of the world; much of the diversity of OECD agricultures can be captured in another couple of dozen farming systems.

FIGURE 2. DISTRIBUTION OF MAJOR FARMING SYSTEMS ACROSS DEVELOPING REGIONS

See Dixon *et al.* 2001 for a full description.

FARMING SYSTEMS IN DEVELOPING REGIONS



DRIVERS OF THE CHANGE
- Population
- Resources, climate
- Energy
- Science, technology
- Markets
- Institutions, policies
- Information

Source: Dixon et al., 2001, www.fao.org/farming systems/

These farming systems can be grouped into the following eight classes in both developing and developed countries:

- Irrigated farming systems, embracing a broad range of food and cash crop production, often for sale;
- Wetland rice based farming systems, dependent upon monsoon rains supplemented by irrigation;
- Rainfed farming systems in humid areas of high resource potential, characterised by a crop activity (notably root crops, cereals, industrial tree crops – both small scale and plantation – and commercial horticulture) or mixed crop-livestock systems;
- Rainfed farming systems in steep and highland areas, which are often mixed crop-livestock systems;
- Rainfed farming systems in dry or cold low potential areas, with mixed crop-livestock-tree and pastoral systems merging into sparse and often dispersed systems with very low current productivity or potential because of extreme aridity or cold;
- Dualistic (mixed large commercial and smallholder) farming systems, across a variety of ecologies and with diverse production patterns;
- Coastal artisanal fishing, often with mixed farming systems; and
- Urban-based agriculture

Perennials offer different advantages within each of these system categories, as examined in the next section.

FITTING PERENNIALS INTO FARMING SYSTEMS

In farming systems, many perennials foster nutrient cycling, reduce wind effects, curtail soil erosion, and improve the micro-climate. Trees represent a class of farm asset that can be liquidated for capital (a 'bank') in times of need – which parallels the narrative around livestock as another easily saleable class of asset. Such asset accumulation is extremely critical to smallholders because many lack access to formal financial markets. Also, the inclusion/expansion of perennial grains or woody perennials in farming systems is a form of income and asset diversification that enhances livelihood resilience and reduces risk. Diversification of farm household activities is a very effective aspect of poverty escape strategies for farm households in many different farming systems, and is often more effective and dependable than intensification. Many perennials offer multiple products, which is an aspect that is particularly attractive to smallholders. For example, perennial rice could produce grain, forage and ecosystem services (e.g. carbon, reduced water erosion). Similarly, agroforestry trees may simultaneously provide fodder, fuelwood energy for the household and/or for sale, construction material, and ecosystem services in addition to high-value products that are produced for consumption and sale.

The production constraints and opportunities in farming systems are rapidly changing, with urbanizing markets, climatic variability and labour shortages growing in importance. Perennials are critical for both capturing new opportunities and for overcoming these constraints. Market constraints are generally declining for smallholder farming populations as infrastructure gradually improves and national, regional and global markets grow. This plays to the advantage of tree products. Perennial grains will reduce field labour requirements, and thus reduce women's labour burden.

We will now examine the present and future role of perennials in the generic classes of global farming systems, as summarized in Table 1.

TABLE 1. PRESENT AND POTENTIAL ROLES OF PERENNIALS IN DIFFERENT FARMING SYSTEMS CLASSES

CLASS OF FARMING SYSTEM	ROLES OF PERENNIAL GRAINS	ROLES OF PERENNIAL PASTURES	ROLES OF WOODY PERENNIALS
Irrigated farming systems, embracing a broad range of food and cash crop production, often for sale.	Limited role until perennial grains' agronomic performance is equivalent to annuals.	Role as a break crop or where livestock (especially dairy) is profitable.	Major role as high-value irrigated vines and fruits; significant roles along bunds, canals and access tracks; major role as intercrops (e.g. wheat-poplar systems), potential role as fertilizer trees and green manures, e.g. Faidherbia, Tephrosia.
Wetland rice based farming systems, dependent upon monsoon rains supplemented by irrigation.	Limited role until perennial grains' agronomic performance is equivalent to annuals.	Limited role for perennial pastures – but significant role for annual forages.	Significant role along bunds, canals and access tracks; high-value trees on mounds within fields (e.g. fruit crops), potential as fertilizer trees and green manures, e.g. *Faidherbia, sesbania*.
Rainfed farming systems in humid areas of high resource potential, characterised by a crop activity (notably root crops, cereals, industrial tree crops – both small scale and plantation – and commercial horticulture), or mixed crop-livestock systems.	Potential role for dual purpose grain/grazing, or as intercrop in tree crops.	Major role for pastures in livestock and crop-livestock systems.	Major role as a diverse range of agroforestry systems for production of fruits, medicinals and neutraceuticals, export commodities (e.g. coffee), high-quality fodder, timber and pole production, fertilizer tree integration.
Rainfed farming systems in steep and highland areas, which are often mixed crop-livestock systems.	Major role for triple purpose erosion control, grazing and grain especially on steep slopes where annual crop establishment would not be sustainable, e.g. p-rice, p-sorghum.	Major role for pastures in livestock and crop-livestock systems in order to control erosion.	Major role for high value trees, timber, and forage trees and shrubs in livestock and crop-livestock systems, for sustainable production systems on steep land evolving out of unsustainable annual cropping, controlling soil erosion and degradation.

CLASS OF FARMING SYSTEM	ROLES OF PERENNIAL GRAINS	ROLES OF PERENNIAL PASTURES	ROLES OF WOODY PERENNIALS
Rainfed farming systems in dry or cold low potential areas, with mixed crop-livestock and pastoral systems merging into sparse and often dispersed systems with low productivity or potential because of extreme aridity or cold.	Potential role for double purpose salinity management and grain production, e.g. p-wheat.	Major role for pastures in livestock and agropastoral systems in order to make best use of available moisture.	Fundamental role as evergreen agriculture in dryland crop-livestock systems to enhance fodder production, improve crop yields through improved nutrient cycling, water holding capacity and buffering microclimate, reducing production vulnerability and increasing resilience. Diversification with high-value fruits, gums, etc. Special role of farmer-managed natural regeneration of Faidherbia and similar trees.
Dualistic (mixed large commercial and smallholder) farming systems, across a variety of ecologies and with diverse production patterns.	As above, depending on the agro-ecology and economic environment.	As above.	As above.
Coastal artisanal fishing, often mixed farming systems.	Potential role in harsh sandy environments where well established roots enable perennials to exploit deeper water.	Limited role because of frequently harsh agro-ecologies and prevalence of fish as an alternative protein source.	Major role for diverse home garden and agroforest systems of fruit, nut and forest species in coastal fishing-based environments.
Urban based, including peri-urban agriculture.	Limited role.	Limited role because of high value of land – thus other feeds used for livestock.	Major role for fruits for cash sales.

IRRIGATED FARMING SYSTEMS, EMBRACING A BROAD RANGE OF FOOD AND CASH CROP PRODUCTION, OFTEN FOR SALE

Farmers in the world's large-scale irrigated systems have generally been pursuing a strategy of crop intensification to maximize crop production through deploying the most advanced genetics, fertilization, pest management, and water management practices available. In areas where these technologies have been exploited to their fullest, the yield gap has been largely closed and only incremental gains are foreseen from intensification. Thus, their attention has been turning to reducing labour costs and to exploring ways to diversify production of enterprises that can provide new and more lucrative opportunities for income gains and income stability. The introduction of perennial varieties of their annual crops (rice, wheat, maize, etc.) could potentially help them achieve reduced labour and other production costs, when and if, agronomically superior varieties become available. With the possible exception of rice, these possibilities appear to be decades away.

Perennial forages play a niche role in some irrigated systems, often underpinning dairy farming and occasionally the fattening of ruminants. For example, berseem clover is a common irrigated forage in the Nile Delta or Wadi Haramout in Yemen. Irrigated grasses and alfalfa are grown for similar purposes in the United States and Australia, but are not yet widespread in irrigated systems in developing countries.

In the meantime, many irrigated farmers, particularly small-scale producers in the tropics, are avidly exploring enterprises that will diversify their income streams, and reduce their current levels of risk dependency on one or very few irrigated crops. This has led to the integration of higher-value crops in their irrigated systems, including fruit trees, vegetables and the like, often partially replacing their irrigated food crops with these alternatives. There has also been a trend toward growing trees for lumber, roundwood for veneer, construction poles, and other wood products.

One example of this has been the trend toward producing tree enterprises on irrigated land in northern India, where considerable areas of irrigated land has now been shifted into wood production. In some systems, timber trees such as poplar have been integrated in irrigated wheat production, improving the land equivalent ratios on the farm, and the overall annual income generated from the land. And in many other areas of the tropics, irrigated land has been shifted into high-value fruit tree production, particularly in countries where the local demand for fruits has increased and/or export markets have opened up, such as in the Sahel. These trends are accelerating in some countries.

Another opportunity that farmers have is to make better use of the non-irrigated portions of their land in the surroundings of irrigated fields. Increasingly, these portions of the farm (roadways, dikes, pathways, and unirrigated corners) are being planted with trees for asset-building, fruit production, environmental amenity, and windbreak microclimate functions to reduce crop water stress. The latter is a particularly important adaptation to the increased temperatures and longer and more severe drought events that are being observed in many areas. The role of perennials in micro-climate buffering will become a major area of interest in climate change adaptation in the future.

WETLAND RICE-BASED FARMING SYSTEMS, DEPENDENT UPON MONSOON RAINS SUPPLEMENTED BY IRRIGATION

Wetland rice systems are a class of irrigated systems where the land is waterlogged and/or under shallow flooding for a portion of the year; such agro-ecosystems are eminently suitable for wetland rice cultivation which has great cultural value in much of Asia. As noted above, the introduction of perennial rice might reduce labour and other production costs, but agronomically superior varieties are not yet available and so we do not anticipate widespread use of perennial rice in wetland rice systems in the near future. The availability of markets for milk and meat have provided incentives for the limited adoption of annual forages in some locations, but there seem to be weaker incentives for the adoption of perennial grasses or leguminous forages.

Naturally, such ecological conditions present farmers with considerable challenges in introducing and managing perennials directly in their rice fields. Nevertheless, opportunities abound. For example, innovative rice farmers in Indonesia, Thailand and Viet Nam are introducing high-value fruit trees directly into their irrigated fields by constructing mounds of soil in a grid pattern that enable them to culture fruit trees and avoid waterlogging the trees while providing them with a highly favourable rooting environment for fast and vigorous growth. This is a variation of the traditional bed and ditch system of rice-growing that became popular in Bangladesh and Indonesia as a means to use waterlogged rice soils for crop and tree diversification.

Many tree species have an inherent genetic degree of waterlogging tolerance. This has provided the basis for the selection of species, particularly timber, fodder and fuelwood-bearing trees that can be produced very successfully in rice fields, particularly in systems where the land is only waterlogged for less than half the year. Bangladesh has been a leader in the testing and incorporation of such species into rice production systems. Vigorous pruning of the trees during the rice-growing season yields fuelwood and fodder while minimizing competition for light, nutrients and water with the rice crops, thus achieving substantial overall income benefits.

Agroforestry trees are increasingly being deployed to reduce waterlogging and salinization of soils in the vicinity of irrigation canals with blocked drainage systems. Rice scientists are also observing that in many situations, excessive soil compaction limits the farmers' flexibility in the preparation of their rice fields for direct-seeding and limited irrigation regimes (Buresh, personal communication. 2013). This has stimulated interest in the possible role of trees in creating soil physical conditions that would enhance the success of these water-saving practices by enhancing field drainage conditions, particularly during land preparation. The choice of tree species with the appropriate rooting dynamics to provide this service role along with the provision of income-generating products could be a suitable approach to overcoming this drainage constraint. Further research in this area is anticipated.

Rainfed and partially irrigated rice-based systems are commonly prone to highly variable yields due to drought stress and flooding events. Thus, cash investments in inorganic fertilizer use, is a risky proposition for smallholder farmers facing these constraints over a huge proportion of the world's ricelands. Practices that would enhance the provision of biological fertilization with minimal labour inputs would be of substantive value in these situations.

On the rice-growing floodplains of the Senegal River, farmers have maintained a fairly dense population of the native tree species *Faidherbia albida*, which is indigenous to these environments. It is a highly tolerant to waterlogging, nitrogen-fixing species that displays reverse phenology, meaning that it is dormant during the rice-growing season, producing minimal shade. These observations have led to the hypothesis that such a compatible species might be the basis for a transformative type of rice-tree production system that would provide an abundant source of biofertilizer (particularly nitrogen) as it sheds its leaves at the beginning of the rain season, and would provide a source of fuelwood and fodder during the dry season, sustaining ruminant livestock in the system.

Rice production systems have always been particularly non-diverse crop production systems because of their unique hydrological situation. But clearly there is ample opportunity to foresee the diversification of these systems through the incorporation of a wide range of perennial options if researchers and extensionists were to pay more attention to the range of farmer innovations that have already pointed the way toward their future transformation.

RAINFED FARMING SYSTEMS IN HUMID AREAS OF HIGH RESOURCE POTENTIAL

Agricultural practices like agroforestry, introducing hedges, low and no tillage and cover crops have an important potential to increase carbon sequestration in rainfed farming systems. Aertsens *et al.* (2013) found that this would correspond to 37 percent of all CO_2-equivalent emissions in the EU in 2007. They found that the introduction of agroforestry was the measure with the highest potential to sequester carbon in European agriculture. Its potential was estimated to be 90 percent of the total sequestration potential of the various practices studied. Taking account only of the value for climate change mitigation, they found that the introduction of agroforestry is estimated to have a value of ↱282/ha in 2012, and that this will gradually increase to ↱1 007/ha in 2030. This implies that there is a very large potential benefit for society in general and for the agricultural sector in particular. At the European level, during the past few years, policy makers have recognized the important benefits of agroforestry, and rural development programmes some European countries now support farmers to introduce agroforestry. But the current level of support is still only a small fraction of the societal value. Aertsents *et al.* (2103) posited that if this value would be fully recognized by internalizing the positive externality, agroforestry will be introduced to a very large extent in the next decades, in Europe and the rest of the world, and that this will dramatically change rural landscapes.

In Africa, the Maize-Mixed Farming System is a dominant one, extending over much of eastern and southern Africa. It has a greater agricultural population and more poverty than any of the other farming systems in Africa, and serves as the food basket as well as driver of agricultural growth and food security in the region. Conservation agriculture (CA) is currently being promoted at a major scale in African maize-growing systems. CA involves minimum soil disturbance, crop residue retention, and crop rotation.

However, the uptake of CA in Africa, and in the rainfed upland areas of Asia, has been modest so far. The short-term advantages observed where CA is currently practiced are earlier planting to enable better use of seasonal rainfall, and increased rainwater conservation in the soil to better tide crops over during drought periods (Rockstrom *et al.* 2009). But there are a number of unique constraints to smallholder adoption of CA that are retarding its more rapid uptake. Most important among these are competing uses for crop residues where livestock production is common, inadequate biomass accumulation of cover crops in the off-season, increased labour demands for weeding when herbicides are not used, variable yield results across soil types, and the need for adequate application of organic and inorganic nutrients.

Recently, the CA and agroforestry research and development communities recognized the value of integrating fertilizer trees and shrubs into systems of 'conservation agriculture with trees' (CAWT). These enhance both fodder production and soil fertility (FAO, 2010, FAO, 2011). Practical systems for intercropping fertilizer trees in maize farming have been developed and are now being extended to hundreds of thousands of farmers in Malawi and Zambia (Ajayi *et al.* 2011; Garrity *et al.* 2010). The portfolio of options includes intercropping maize with *Gliricidia sepium*, *Tephrosia candida*, pigeon peas or forage legumes, or using trees such as *Sesbania sesban* as an improved fallow.

One particularly promising system is the integration of the *Faidherbia albida* in crop fields at a 10 m by 10 m spacing. *Faidherbia* is an indigenous African acacia that is widespread on millions of farmers' fields throughout the eastern, western, and southern regions of the continent. It is highly compatible with food crops because it is dormant during the rainy season, and it exhibits minimal competition, while enhancing yields and soil health (Barnes and Fagg, 2003). Several tonnes of additional biomass can be generated annually per hectare to accelerate soil fertility replenishment, provide additional livestock fodder. Numerous publications have recorded increases in maize grain yield when it grown in association with Faidherbia, ranging from 6 percent to more than 200 percent (Barnes and Fagg, 2003), depending on the age and density of trees, agronomic practices used, and the weather conditions. These CAWT systems are a type of Evergreen Agriculture in which trees are managed as an integral element of crop fields (Garrity *et al.* 2013).

Of course, commercial tree crops such cocoa, coffee, rubber and oilpalm are concentrated in humid rainfed farming systems such as in coastal West Africa, Malaysia of Kalimantan, Indonesia. Often the tree crop is grown as a monoculture, but increasingly farmers are realizing additional income by inter-cropping during establishment with annual food or high value crops (e.g. vegetables) or perennials pastures and ground cover during later years.

In general, incorporating trees into crop farming may confer sustainability benefits through ecological intensification. They may increase the resilience of the farm enterprise to climate change through greater drought resilience, and they sequester more carbon. Conventional CA systems tend to sequester a maximum of 0.2–0.4 tonnes C/ha/yr. CAWT systems may accumulate carbon both above and below-ground in the range of 2–4 tonnes C/ha/y, roughly an order of magnitude higher than with CA alone (Garrity *et al.* 2010). This is particularly true for systems incorporating fertilizer trees such as Faidherbia or Gliricidia (Makumba *et al.* 2007). Consequently, there is considerable interest in the development of reward systems to channel carbon offset payments from developed countries to stimulate more carbon sequestration in African food crop systems while simultaneously enhancing the livelihoods of smallholders and the environment. These investments will encourage development pathways resulting in higher carbon stocks at a whole landscape scale.

CAWT systems are now attracting considerable research and extension attention. Their success will depend on the use of a wider range of tree species for varied agro-ecologies, higher quality tree germplasm, better tree seed dissemination systems, and further improvements in tree propagation and establishment methods. The optimum tree densities for different CAWT systems have yet to be fully understood, and the best practices in exploiting the soil fertility synergies between organic and inorganic nutrient sources need to be elucidated. Targeting and scaling-up methodologies deserve particular attention. These need to be supported by work to reverse detrimental policy frameworks in some countries that may discourage farmers from cultivating trees on farms. Also, active farmer organizations have always been instrumental in the development and spread of CA. Thus, the growing interest in Landcare for grassroots mobilization in Africa and Asia can provide a particularly suitable approach for the engagement of farming communities in the refinement and spread of CAWT.

RAINFED FARMING SYSTEMS IN STEEP AND HIGHLAND AREAS

This farming system has the most to gain from increased perennialization, associated in large measure with the ability of perennials to provide surface cover and to drastically reduce erosion. Hill farming systems in southern China and the Mekong might become the first beneficiaries from perennial rice, producing both biomass and grain while stabilizing the ecosystem and reducing labour requirements in areas with strong market demand for ruminant products. In this role, perennial rice might compete with perennial forages which are increasingly being intensively managed in sloping land agriculture as in the Philippines and much of south-east Asia. Of course, similar roles might be identified in sub-tropical and sub-tropical and temperate zones.

Subsistence annual cropping systems have spread to many steeply sloping lands in the tropical developing countries as a consequence of poverty, unemployment, and the shortage of land. Continuous cropping on steep lands generally results in enormous rates of soil erosion and rapid land degradation. Perennial crop systems have proven to be a much more sustainable land use in these ecosystems. Tree crops such as rubber, oil palm, and cocoa have been expanding rapidly on these sloping lands during recent decades, particularly in Southeast Asia.

In eastern Africa the farming systems have also come to be dominated by perennial crops, particularly coffee, tea and cooking bananas (Garrity, 2012). These areas now support some of the highest rural population densities in sub-Saharan Africa. They also exhibit some of the highest agricultural potential. They have been a natural experiment in the interaction between population growth, declining farm sizes, and the intensification of farming systems., as sustainable intensification runs up against extreme limits to minimum farm sizes, and as well as the possibilities and limits of farming systems commercialization. They are now characterized as permanent systems and fallowing for soil regeneration is no longer possible (Carswell, 2002). The liberalization of markets in the late 1990's now offers a principal pathway for further intensification of these farming systems.

RAINFED FARMING SYSTEMS IN THE DRYLANDS

Because of the moisture limitations and climatic variability in semi-arid areas, perennial crops, pastures and trees have a natural advantage and often produce more biomass than annual plants (the converse can be true of arid zones). Sorghums and millets are common food grain crops in the tropical drylands, yet often fail because of establishment drought. Hence perennial sorghum (or millet) might be attractive to many risk-averse smallholder farmers. The farming systems of the drylands generally feature the integration of crops and livestock, with crop residues representing and important source of fodder for ruminants. In this context, dual purpose grain/graze cereal or legume crops would have advantages; and so too a stay-green trait for deep-rooted perennials which could maintain forage quality in the early part of the dry season.

Of course perennial natural pastures are the mainstay for livestock industries in most dryland areas. The ecosystem services from rangelands are often under-estimated, not least erosion control and carbon sequestration. However, the importance of small scale irrigation in the drylands is often overlooked. Such highly productive patches stabilize farm-household livelihoods, whether used for crop or forage production – and for the latter perennial grass and legume forages, mixed or as a monoculture, would have many advantages.

The retention of trees in dryland crop fields in the tropics has been a widespread traditional practice in semi-arid areas. In the Sahelian region these agroforestry parkland systems became common as agriculture gradually intensified (Boffa, 1999). The trees are an integral part of the agricultural system, providing food, fuel, fodder, medicinals, wood for buildings, cash commodities, as well as contributing to soil fertility, water conservation, and environmental protection. However, demographic, economic, environmental and social developments during the past 40 years have put pressure on traditional land-use systems, and concerns have intensified about the steady degradation of land health in the semi-arid dryland agricultural systems in the tropics. This has turned attention to the ways that trees and shrubs can be more successfully integrated into food crop systems on a larger scale, in order to regenerate the soil health, increase annual crop yields, and diversify livelihoods, building on the knowledge and practices of dryland farmers themselves.

A globally relevant model of positive action has evolved in Niger. Since 1985, more than a million rural households in Niger have protected and managed the natural regeneration of native trees, growing in farm fields across 5 million hectares. Nigerien farmers have added approximately 200 million additional trees across agricultural landscapes, which have directly contributed to the increased production of about 500 000 tonnes of grain per year, an amount sufficient to feed an additional 2.5 million people (Reij *et al.* 2009). The United States Geological Survey also recently mapped 450 000 hectares of newly created agroforestry parkland in the Seno Plains of Mali (Tappan, 2012). Farmers in Zambia and Malawi are also increasing the protection and management of trees on farms and increasing adoption of intercropping of nitrogen-fixing species, including the native tree *Faidherbia albida*. It is estimated that currently about 500 000 Malawian farmers have Faidherbia trees on their farms (Garrity *et al.* 2010). The majority of these trees grew through assisted natural regeneration of seedlings that emerged in farmers' fields.

POLICY CONSIDERATIONS

It is worthwhile considering the policy and institutional settings which will influence the spread of perennials and benefits therefrom. Of course, starting about 10 000 years ago annual crops began to progressively replace perennial food plants including grasses, tubers and fruit trees.

Much annual food crop production is supported, in principle or in practice, by public subsidies, e.g. seed, fertilizer and machinery subsidies. Thus, for widespread adoption of perennial food crops a 'level playing field' would be required – either through reduction of input subsidies or compensating subsidies to perennial crop, pasture and tree adoption. Of course, public support to perennials R&D would be important (and relatively easy to justify because perennials offer prospects of greater eco-system services than annuals). It should be recognized that many annual crops with high input levels provide incentives for private sector involvement, whereas perennial crops would generally require less management of seed/planting material and inputs.

Because of the complexity of farming systems incorporating perennials, research managers should support participatory research and development methods. Such methods might lead naturally to enrichment of farming systems rather than wholesale replacement of annual crops.

CONCLUSION

Perennials are increasingly appreciated as playing a major role in agricultural diversification, risk management and mitigation. This brief survey has highlighted some of the innovative ways that perennials are being deployed in major farming systems around the globe, and the rationale for this trend. Clearly, the potentials, resource pressures and intensification pathways vary across the different types of farming systems (Dixon *et al.* 2001). It is at the level of the farm system that the trade-offs between food security, livelihoods and adaptation to climate change become apparent. And the relative importance of poverty escape pathways varies across farming systems. For example intensification and enterprise diversification dominate poverty reduction in high potential pathways but off-farm income and exit from agriculture are important in low potential systems. These differences in pathways and tradeoffs should be reflected in the design of sustainable intensification research programmes and policies.

Growing concerns about how agriculture will adapt to climate change, how food security can be enhanced, extreme poverty eliminated, and how land degradation processes will be reversed, have accelerated efforts to advance the roles that woody perennials and perennial crops will play in the future of farming. Their role will be transformative over the medium to long term, but they represent opportunities that are often at odds with conventional path-dependent thinking. Research investments and agricultural policy continue to be overwhelmingly dominated by short-term interests. And the enormous opportunities that the perennialization of agriculture are opening up have yet to be translated into commensurate research and policy attention.

REFERENCES

Aertsens, J., de Nocker, L. & Gobin, A. 2013. Valuing the carbon sequestration potential for European agriculture. *Land Use Policy*. 584-594.

Asbjornsen, H., Hernandez-Santana, V., Liebman, M.Z., Bayala, J., Chen, J., Helmers, M.J. & Ong, C.K. 2013. Targeting perennial vegetation in agricultural landscapes for enhancing ecosystem services. *Renewable Agriculture and Food Systems*: (Available at http://lib.dr.iastate.edu/abe_eng_pubs/374).

Chen, S. & Ravallion, M. 2012. *An update to the World Bank's estimates of consumption poverty in the developing world*. Briefing note, Development Research Group, World Bank, Washington, USA.

Dixon, J., Gulliver, A. & Gibbon, D. 2001. *Farming systems and poverty. Improving farmer's livelihoods in a changing world*. FAO & World Bank, Rome, Italy & Washington DC, USA

FAO, WFP. & IFAD. 2012. *The state of food insecurity in the world 2012. Economic growth is necessary but not sufficient to accelerate reduction of hunger and malnutrition*. FAO, Rome, Italy.

Farquharson, E., Abadi, A., Finlayson, J., Ramilan, T., Li Lui, D., Anwar, M., Clark, S., Robertson, S., Mendham, D., Thomas, Q. & McGrath, J. 2013. *Everfarm ® – Climate adapted perennial-based farming systems for dryland agriculture in southern Australia*. Final Report. CSIRO. GRDC.

Fuglie, K. & Wang, S.L. 2012. New evidence points to robust but uneven productivity growth in global agriculture. *Amber Waves.* 10(3): 1-6.

Garnett, T., Appleby, M.C., Balmford, A., Bateman, J., Benton, T.G., Bloomer, P., Burlingame, B., Dawkins, M., Dolan, L., Fraser, D., Herrero, M., Hoffmann, I., Smith, P., Thornton, P.K., Toulmin, C., Vermeulen, S.J. & Godfray, H.C.J. 2013. Sustainable intensification in agriculture: Premises and policies. *Science.* 341(5 July): 33-34.

Garrity, D.P., Akinnifesi, F., Ajayi, O.C., Weldesemayat, S.G., Mowo, J.G., Kalinganire, A., Larwanou, M. & Bayala, J. 2010. Evergreen Agriculture: a robust approach to sustainable food security in Africa. *Food Security*. 2: 197–214.

GO-Science. 2011. *Foresight. The Future of Food and Farming: Challenges and choices for global sustainability*. The Government Office for Science, London, UK.

Godfray, C., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M. & Toulmin, C. 2010. Food security: the challenge of feeding 9 billion people. *Science.* 327: 812–818.

IFPRI. 2013. *Global Hunger Index, The callenge of hunger: the challenge of hunger. Building resilience to achieve food and nutrition security*. IFPRI, Washington, USA.

Lee, D.R. & Barrett, C.B. 2001. *Tradeoffs or synergies? Agricultural intensification, economic development and the environment*. CABI, Wallingford, UK.

Keating, B.A., Carberry, P.S., Bindraban, P.S., Asseng, S., Meinke, H. & Dixon, J. 2010. Eco-efficient agriculture: concepts, challenges and opportunities. *Crop Science.* 50(S1): 109-119.

Reij, C., Tappan, G. & Smale, M. 2009. *Agroenviromental transformation in the Sahel: Another kind of 'Green Revolution'*. International Food Policy Research Institute, Washington, USA.

Tilman, D., Balzer, C., Hill, J. & Befort, B.L. 2011. Global food demand and sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences of the United State of America.*. 108(50): 20260-64.

Waddington, S.R., Li, X., Dixon, J., Hyman, G. & de Vicente, C.M. 2010. Getting the focus right: production constraints for six major food crops in Asian and African farming systems. *Food Security*. (March 2010).

World Bank. 2008. *World Development Report 2008*. Agriculture for Development. World Bank, Washington, USA.

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PERENNIAL POLYCULTURES: HOW DO WE ASSEMBLE A TRULY SUSTAINABLE AGRICULTURAL SYSTEM?

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ABSTRACT

Population growth and demand for food continues to place pressure upon agriculture to provide for mankind. Primary staple production is currently from annual crop species. Herbaceous perennial species for grain and other food products have not been rigorously pursued. Current interest and research into the development of herbaceous perennial species for food is providing new options for food production systems. Development of perennial species will provide the cornerstones for perennial polyculture development. Production challenges including weed competition and fertility requirements may addressed by perennial polyculture. Utilization of multiple species has been demonstrated to provide greater DM productivity by utilizing the entire growing season. Issues concerning synchronization of production and harvest however may not be easily resolved. Historically selection of perennial grasses species for seed production has most often failed to translate into consistent enhanced productivity at the field scale. Current selection methodology and nursery design are most likely inadequate to address field level productivity issues. Competitive nurseries are suggested to provide field level gains at both the mono and polyculture levels. Landscape-wide utilization of narrow genetic cultivars will lead to divergent communities and reduce reliability of production. Initial development and deployment of perennial grains and oilseeds would be enhanced by the utilization of greater diversity within the crop species. Utilization of companion species will aid in many issues related to sustainability, e.g. N_2 fixation, weediness. Initial economic utilization of perennial grains and oilseeds and perennial polyculture are linked to animal production.

Keywords: perennial grain and oilseeds, selection, competition, diversity, companion species

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Greater demands for food production, for efficiencies in food production and for sustainability of food production systems are required to meet the needs of an ever-growing population.

Herbaceous perennial species are only recently receiving favourable consideration for grain and oilseed production. Whether owing to the size of the seed, or the ease of establishment and combined with relatively quick production, annuals have been preferred and therefore have garnered almost all of the effort and resources for improvement. Breeding and selection of herbaceous perennials for their seed crops has also received very little effort over the millennia of agriculture. Where improvement in seed productivity has been attempted, seed yield improvement of herbaceous perennials has frequently not experienced great success.

NATURALIZED PRODUCTION

“Production agriculture with its ecosystems simplification, pesticide and fertilizer use, and emphasis on yield, often appears to be at odds with conservation biology.” (Banks, 2004). Potential for perennial polyculture to bring agriculture and conservation biology closer may be demonstrated in the ecology of natural production systems such as prairies (Glover *et al.* 2010).

Inputs for controlling environmental factors such as water stress via irrigation have negative impacts at the landscape level (Pataki *et al.* 2011). Reduction in anthropogenic impacts of agriculture may be accomplished through the use of perennials for bioenergy (Georgescu *et al.* 2011) and therefore, by extension, herbaceous perennials as grain, oilseed and potentially as other types of crops. Perennial monocultures for bioenergy are still subject to yield fluctuations owing to environmental conditions, despite adequate agronomic practices (Tulbure *et al.* 2012). Grasslands, nature’s polycultures, however are seen as important for carbon sequestration (O’Mara, 2012).

Monoculture seed production of herbaceous perennials, while allowing for many environmental benefits (depending upon inputs), has some shortcomings. Shortcomings include added fertility, weed control, insect control and a single, defined growth period. All of the above may be addressed through polyculture.

POLYCULTURE

Hunter-gatherer societies in North America had long been passive participants in natural polycultures with their infrequent harvests across wide areas (Kuhnlein and Turner, 1991). However no active polycultures of desirable species are reported, although some groups practiced monoculture agriculture (Kuhnlein and Turner, 1991). We currently practice polyculture in home vegetable, herb and ornamental gardens and in our production of forages for animal feed (e.g. Picasso *et al.* 2008; Wiltshire *et al.* 2010). Pastures and rangelands provide resource conservation, biodiversity enhancement and ecosystem preservation (Wiltshire *et al.* 2010). Polyculture establishment of herbaceous perennial species for large-scale food or feed production will be a new endeavour for humans. Perennial polycultures, where coupled with animal production, are envisioned to require little if any outside applications of nutrients or pest management materials (Glover *et al.* 2010) and the benefits to sustainability should surpass those which have been attributed to organic systems (Lammerts Van Bueren *et al.* 2002) owing to the potential to resist short- and long-term variations in the growth environment.

The Land Institute with its concept of natural systems agriculture has been the recent champion of perennial crop breeding (Jackson, 2002; DeHaan *et al.* 2005; Cox *et al.* 2006, 2010). Once perennial grain and oilseed crops have been developed, a logical next step will be polyculture. Polyculture proposes the annual harvest of potentially several crop species cropped together in each year (Jackson, 2002) and emphasizes sustainability. No single species predominates across the landscape (MacDougall *et al.* 2013) (Figure 1). Even areas predominated by invasive species allow niches for native species to persist (Gilbert and Levine, 2013).

Many herbaceous perennial forage crop species are produced under bi- or polyculture conditions. In experiments where up to sixteen and twenty-four species were seeded (Tilman *et al.* 2001, 1996, respectively), as the number of plant species seeded increased, higher above-ground biomass productivity was realized and soil nitrogen use by the plant community was more complete. DeHaan *et al.* (2009) however showed that if perennial biomass productivity is the sole aim, bi-cultures of a grass and legume are as productive. Seed productivity of perennials, where species maturity and reproductive productivity are major concerns was not addressed. Mixtures of grasses and legumes were found to provide increased DM production over a number of years (Sturludóttir *et al.* 2013). Similar efforts will be required for both bi- and polyculture for food.

Monoculture production of either annual or perennial species, is subject to appearance of non-intended plants (weeds) (Cattani *et al.* 2009; Sturludóttir *et al.* 2013) (Figure 2).

FIGURE 1. VARIABILITY IN STAND IN A HERBACEOUS PERENNIAL FORAGE SPECIES

FIGURE 2. COMPETITION IN AN HERBACEOUS PERENNIAL SEED PRODUCTION FIELD WITH UNSEEDED SPECIES OCCURRING

Picasso *et al.* (2008) found that as the number of seeded species increased above-ground DM production by weeds decreased. Weeds status however can be an arbitrary assignment to a species. Fletcher (1897) defines weed as: "There are many definitions of the word weed … from a farmer's standpoint …, 'any troublesome or unsightly plant that is at the same time useless or comparatively so'." This statement implies recognition that there are plants whose impacts are either neutral or beneficial, indicating that multispecies communities were known and accepted. Perennial polycultures with their growing- season long growth potential should lead to reduced weed growth and reduced potential yield loss due to reduced weed competition as seen in many annual crops (e.g. Zhao *et al.* 2006). In many perennial seed increase operations, manual removal of troublesome species is required owing to either the lack of an adequate control chemical and/or timing of appearance of the troublesome species with respect to reproductive growth of the desired crop species.

Weinberg's (1975) systems complexity theory hypothesizes that organized complexity (systems) are less random than unorganized complexity (aggregates). Extending Weinberg's (1975) systems complexity theory to monocultures versus polycultures, with the lack of system complexity in monocultures (e.g. relative genetic uniformity, single species), random events or factors entering the system and not under the control of the producer (e.g. climate, insects, weeds, disease) may have major impacts on the system (e.g. year to year variation in yields).

Human intervention in ecosystems may lead to the loss of redundancy in native species diversity (MacDougall *et al.* 2013). Polyculture systems should provide sufficient system plasticity to adapt to variability in growth environment and allow for adequate production. Components of total productivity will vary from year to year and from location to location (see Picasso *et al.* 2008). Included in this complexity and potential interactions, is the reduced ability to quantify effects of individuals due to the interactions (Weinberg, 1975). These interactions are the most critical components of multispecies (polyculture) systems (Chen and Welter, 2005; Dray *et al.* 2012).

Construction of a community that enhances the productive capacity of crop species or several crop species is possible. Companion species however, should not restrict crop production, or if a reduction occurs, the companion species contribution should be to a future year's productivity or to sustainability. Therefore, productivity must be evaluated over the life-span of plantings and include benefits accrued to the land unit (e.g. N_2 fixation, soil organic matter, weed control) and include ecosystem benefits and not be measured solely by crop yield and market value. Selection of proper species and selection potentially within of all component species will be required to ensure growing-season-long growth for greater sustainability and productivity. Selection within companion species may need to be against uniformity.

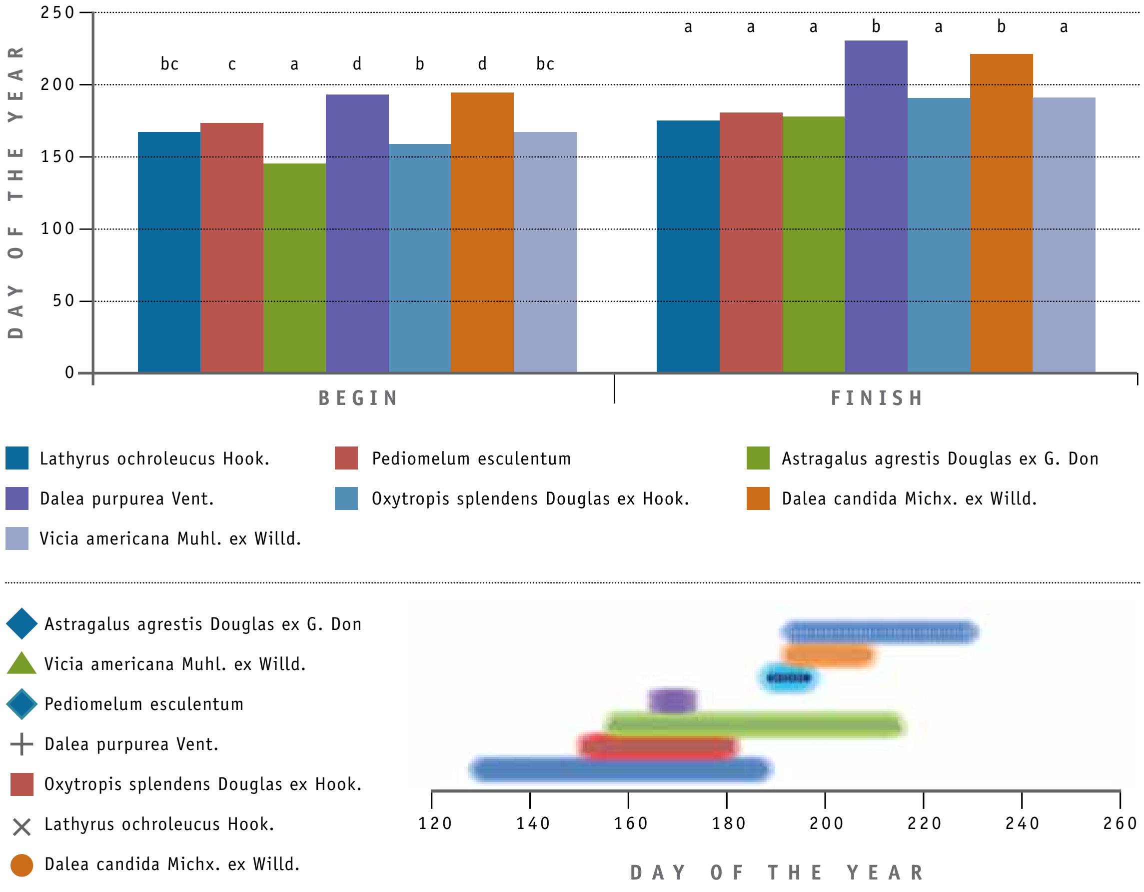
A properly designed perennial system should greatly reduce inputs demands, nutrient losses and the associated environmental impacts (Crews, 2005). Species coexistence depends in part upon temporal variation and therefore polyculture may also buffer impacts of climate volatility (Adler *et al.* 2006). For example, alfalfa (*Medicago sativa*) and stinkweed (*Thlapsi arvense*) are frequently found together in alfalfa seed production fields (perennial seed crop) in Manitoba, Canada (Cattani *et al.* 2009). Overlapping of the reproductive growth and development phases of these species does not occur whereas Canada thistle (*Cirsium arvense*) is relatively synchronized with alfalfa with respect to flowering (Cattani *et al.* 2009) (Figure 2). Canada thistle directly competes with alfalfa during its flowering and pod fill periods and is considered one of the major weed species in alfalfa seed production. The former may be an example of coexistence or concurrence while the latter exemplifies yield competition.

If Weinberg's (1975) theory holds, genetic diversity within a given species must also be broad (greater complexity) in order for that species to be able to appear across a wide swath of the landscape. Vellend (2006) predicts this and Picasso *et al.* (2008) found that the end result of polycultures in both different systems and locations can lead to different production communities. Picasso *et al.* (2008) however, used cultivars or composites for all of their seed sources which may have been too narrow genetically but only if the desired result was a similar species composition across diverse environments. Species diversity has been found in restored grasslands (Helsen *et al.* 2012) and this is most likely due to the occurrence of niches within an ecosystem (MacDougall *et al.* 2013). Selection should aid in the utility of crop and component species. Other factors that have the potential to impact plant biodiversity and persistence include herbivory (Chen and Welter, 2005; Dyer *et al.* 2010; Stein *et al.* 2010), foliar pathogens (Allan *et al.* 2010) and system management (MacDougall *et al.* 2013). Once established, plant recruitment is primarily from vegetative reproductive structures, with seedling establishment contributing very little (Jonsdottir, 1991; Benson and Hartnett, 2006).

Plant systems do not occur in isolation. An estimated 87.5 percent of angiosperms interact with pollinators (Ollerton *et al.* 2011). Cane (2006) reported that *Dalea purpurea* attracted a wide array of pollinating insects throughout its flowering period while Clement *et al.* (2006) list a number of species visiting *Astragalus* and *Onobrychis sp.* Facilitation by common species may allow for pollination success of rare species (Bizecki Robson, 2013). This indicates that selection of a few species that attract diverse pollinators and that have a somewhat overlapping flowering period may be used to provide adequate sustenance for pollinating species and to perpetuate pollinator species across years (Hajjar *et al.* 2008).

We have been monitoring flowering periods of native species for the past four growing seasons at sites around Manitoba. Looking at native legumes, flowering times are relatively consistent across years with respect to their order (Figure 3a). Selection of which species to include that would ensure an overlapping of flowering periods could result in dual purpose species, i.e. species that attract pollinators and are N_2 fixing. Other considerations such as potential toxicity to animals, if post-harvest biomass is to be grazed or fed, must also be considered (e.g. *Oxytropis splendens,* Macdonald, 1974).

FIGURE 3. A) MEAN BEGINNING AND FINISHING OF FLOWERING TIMES OF SEVEN NATIVE LEGUME SPECIES AT SETON, MB, FROM 2010-2013; B) MEAN OVERLAP OF FLOWERING PERIODS OF NATIVE LEGUMES AT SETON, MB, FOR 2010-2013



Breeding of perennial species for use in polyculture will be dependent upon the individual species involved as crop species. If, for example, *Helianthus maximiliani* is bred for uniculm production, yield will likely be dependent upon pollinator availability. Companion species' that can attract pollinators and flower near and/or across the period of *H. maximiliani* flowering will aid in productivity. Companion plant density will need to be weighed against crop plant density to determine the appropriate balance. Pollinators may be brought in from outside the area of production however sustainability is decreased in favour of profit and potentially at the expense of the surrounding ecosystem.

SELECTION

Selection for perennial herbaceous species has primarily been for forage production (Casler and Brummer, 2008). Cultivar development can often be outside of the country of use (e.g. Acharya *et al.* 2013).

Performance in agricultural settings is often very specific. Harlan and Martini (1938) found that few barley varieties were adapted across wide swaths of the landscape. Selection pressure with respect to the genetic uniformity of the crop species requires that it be determined prior to system development. Cultivar development dictates relative morphological uniformity within the resultant cultivar for identification purposes.

One approach for landscape-wide polyculture plantings requires multiple cultivars being developed to the current regulations only to utilize a number of cultivars in an individual planting to increase diversity of the crop species' with the aim of enhancing the crop's ability to withstand stresses (e.g. disease, moisture extremes) across the landscape. Limited selection within a species on important traits (e.g. seed yield and synchronous flowering) yet maintaining genetic diversity could benefit this system by providing adequate plasticity within each species (e.g. Ecovar™, Ducks Unlimited Canada). Utilization of the entire growing season could be achieved by selecting for maturity differences especially within the supporting species. Increasing diversity within component species should allow for greater adaptability, reducing the variation in the composition among communities established at different sites (Vellend, 2006).

How then should selections for perennial species be made? Will plant nursery type impact the utility of the end product in a different system (e.g. monoculture versus polyculture)? Can progress be made and maintained?

If productivity is to be realized from more than a single species, harvest timing must be such that (e.g. in a two crop species system) either one species is harvested prior to the reproductive growth of the second or both species mature simultaneously and/or are harvested simultaneously. Harvestable species may dictate the system utilized as inherent qualities of the species (e.g. shattering) could determine the approach taken. Companion species benefits must be quantified, possibly their contributions via N_2 fixation, attraction of pollinators (e.g.

Dalea purpurea (Cane, 2006)) or the ability to suppress other plant growth that would have a greater negative impact on the harvestable species. Ground cover provision in spring or autumn when the crop species may be relatively non-competitive may be beneficial. If a crop assembly is desired, testing will be required to identify ranges of reproductive effort of the potential member species and then combinations of complimentary maturation timings for sustainability of the system.

Elgersma *et al.* (1994) found that selection in space planted nurseries did not correlate to seed yield in progeny seed rows in perennial ryegrass (*Lolium perenne* L.) while Hayward and Vivero (1984) found similar results for forage yield in perennial ryegrass. Burton and DeVane (1953) inferred that advancement under selection under space planted conditions does not translate to similar increases in forage or seed yield in competitive stands. Genetic improvement for forage yield in perennial forage crops has been relatively poor (Casler and Brummer, 2008) due to the lack of a readily apparent traits and selection against 'quality' traits. Conversely, selection for harvest index in annual crops has allowed for genetic gain for yield, but possibly at the cost of overall fitness (Chen and Welter, 2005).

Seed yield component compensation may be impacting plant reproductive efforts under competitive conditions. Yield component compensation is likely environmental and not genetic and that the sequence of developing traits is important (Adams, 1967). Dofing and Knight (1992) based their proposed model for path coefficient analysis on this premise. Species ability to compensate for yield components (plasticity) will in part be dictated by the reproductive requirements of the species as well as reproductive morphology. For example, requirements for dual induction (Heide, 1994) may limit a plant's ability to recover from a poor autumn regrowth period in the spring if reproductive tiller number is fixed by autumn regrowth (e.g. Cattani *et al.* 1997) or by spikelets consisting of a single perfect floret (Cattani *et al.* 2004).

Stand duration prior to selection will also impact adaptation and persistence. Local adaptation is important and may take up to three years to become evident in a perennial species (Hufford and Mazer, 2012). Selection for harvest index in perennial species may decrease overall stand duration via allocation to sexual reproduction versus perenniating structures and tissues (parent-offspring conflict, e.g. Zhang and Jiang, 2000) given the importance of vegetative reproduction in perennial grasslands (Jonsdottir, 1991; Benson and Hartnett, 2006). A positive correlation between storage (corms) and seed production in *Amphibromus scabrivalvis* was reported indicating that increasing harvest index may not necessarily reduce long-term fitness and survival (Cheplick, 1995). In two *Geranium sp*. it was found that flowering had different effects on the following year's flower production (Ågren and Willson, 1994). Fitness reduction may increase with greater pollination success (Ågren and Willson, 1994) therefore selection for increased fertility and increased harvest index may negatively impact long-term survival of the crop in the field. Therefore, species differences may be such that seed yield progress under selection may impact life history in some species but not others.

"The potential seed yield of forage species is high, whereas realized seed yields are generally low and unpredictable." (Elgersma and van Wijk, 1997), due in part to the lack of importance placed upon seed versus forage production of these species and domestication traits such as shattering resistance. The Fecundity Allocation Premium hypothesis indicates that larger (by mass) species can have a greater range of variation of seed sizes (by mass), however species with larger seeds are more likely to have lower lifetime fecundity (Aarssen, 2005). Simply put larger but fewer seeds. Therefore, selection for larger seeds may reduce seed number per plant. In order to increase seed size and/or number for production purposes, selection for larger plant size should then be required. Or selection for greater allocation to seed mass or increased seed number may be at the expense of perenniating structures. The challenge, at least until adaptation to the growing environment is complete, will be to select for increased seed size and/or seed set and retain sufficient tolerance to "normally expected stress levels" and acknowledge the risk of extreme stresses adversely affecting stand longevity. Selection for increased seed productivity however may reduce the competitive ability of the individual within the community and enhance the diversity.

Schaaf and Rogler (1962) found seed weight highly heritable but not yield in crested wheatgrass. Christie and Kalton (1960) indicated that recurrent selection over inbreeding in *Bromus inermis* and seed weight selection on space plants was effective. Selections for seed yield in tall fescue based on clone materials were correlated to single cross progeny tests however, each parent was cloned 40 times (Thomas and Frakes, 1967). This methodology will greatly increase resources required for a selection programme. Knowles (1977) was successful using space-planted nurseries with intermediate wheatgrass, however the author noted that the moderate creep of the plants and the use of two and three year-old plant stands for selection purposes may have approximated production field conditions.

Selection in space planted nurseries therefore may be successful in perennial crops for both end use and seed production characteristics if related to identifiable characteristics. In creeping bentgrass selection for reduced plant spread resulted in greater tiller density in the intended end use, golf course turf (Cattani *et al.* 1996). These selections were also based upon higher reproductive tiller density for seed production purposes and resulted in higher harvest index values across production years in field studies (Cattani *et al.* 2004). Increased tillering was shown to be related to leaf appearance rates and reduced internode lengths (Cattani *et al.* 2002). Therefore improvements in perennial species for seed production can be made, however fitness traits may be reduced, (e.g. dwarf phenotypes). Three important caveats to the success of the above are: 1) the production region was identified prior to selection; 2) the product was for a monoculture seed production system; and 3) species plasticity may compensate for changes within the growth environment and reduce or nullify selection efficacy.

SELECTION NURSERIES

Plant competition can impact performance, and with polyculture the plant-to-plant interactions may be most important to overall stand performance. Weiner *et al.* (2010) argue for selection under high density and with group selection to increase characteristics for the good of the collective stand. The typical yield improvement approach is to improve an individual's fitness.

Annual species are better suited to the theory that individual fitness is what has been under natural selection, while perennial species have evolved other mechanisms of fitness (i.e. perenniating structures). Therefore selection for individual reproductive fitness may lead to increased productivity in perennials at the cost of perenniating vegetative structures. Care is then needed to balance reproductive methods to ensure long-term persistence of a plant within a stand.

A plant's performance may be dependent upon its neighbours and selection without this competition may impact not only the performance of the species in polyculture but also the overall performance of the polyculture. Selection in competitive nurseries should provide greater performance from all component species. Differences in access to resources as described by Smith *et al.* (2009) (Resource Pool Diversity Hypothesis) could account for differential performance under competitive versus non-competitive selection environments. Callaway and Aschehoug (2000) provide an example of differential impact of root exudates from *Centaurea diffusa* and competition for phosphorus on grasses dependent upon whether *C. diffusa* was from it its' area or origin versus its area of introduction. This is important in that competition for phosphorus was not reduced and may be explained by RPDH, and that selection for reducing allelopathic effects is possible.

A number of factors will impact a community including competition, facilitation and evolutionary processes (Brooker, 2006). The example of *C. diffusa* above illustrates evolutionary processes. *Helianthus maximiliani* (perennial sunflower) is a potential perennial oilseed crop and there are reports that it can be allelopathic (Leather, 1983). If true, selection in competition with *H. maximiliani* will be needed to provide maximization of reproductive effort for co-crop species and supporting species in polyculture.

Selection for monoculture cropping does not translate into optimum production in mixtures and selection under production environment conditions is needed (Wright, 1985). Similarly, Lammerts Van Bueren *et al.* (2002) argue that in order to make progress in developing adapted varieties for organic production selection and evaluation must take place under organic conditions, i.e. the conditions of production environments.

Perennial crop cultivars have lasted long after introduction, (e.g. Kentucky 31 tall fescue released in 1943, Climax timothy in 1947) are still in demand in 2013. Based on current regulations, intermediate wheatgrass (*Thinopyrum intermedium*) could therefore be produced for up to six years as a certified seed crop if seeded with foundation seed (CSGA, 2011). Additional years of seed production would then be considered common seed. Seed (grain) for human or animal consumption does not have to meet seed certification quality standards (for genetic make-up) but would be subject however to end-use quality parameters.

Adoption of new germplasm and the ability maintain desired seedstocks can be dependent upon socio-economic issues as well as agronomic performance (Sperling and Loevinsohn, 1993). Perennial crop species may reduce this through the need to only establish the crop once and make repeated harvests from the area, minimizing the risk of successive poor harvests reducing seedstocks. Additionally, land races may be developed over time, reducing the requirement for breeding for specific regions within the landscape.

CONCLUSIONS

Selection within herbaceous perennials for grain and oilseed production has historically not received great interest. Perennial grain and oilseed crops can be achieved however long-term resources must be available to allow for development. Once individual species have been developed, polyculture will be a logical next step. As development of individual species for enhanced forage production has not shown good correlation to production in bi- or polycultures for forage, breeding for polyculture production then will involve selection under different conditions than we currently employ for individual species improvement. Companion species will be required to contribute to sustainability of the system. Profitability of the crop stand will need to be measured by parameters other than simply crop market value to ensure fair comparison of systems and crops.

REFERENCES

Aarssen, L.W. 2005. Why don't larger plants have proportionately bigger seeds? *OIKOS*. 111: 199- 207.

Acharya, S.E., Sottie, B., Coulman, A., Iwaasa, T., McAllister, Y.I., Wang, & Liu, J. 2013. New sainfoin populations for bloat-free alfalfa pasture mixtures in western Canada. *Crop Science*. 53: 2283-2293.

Adams, M.W. 1967. Basis of yield component compensation in crop plants with special reference to the field bean. *Crop Science*. 7: 505-510.

Adler, P.B., HilleRisLambers, J., Kyriakidis, B.C., Guan, Q. & Levine, J.M. 2006. Climate variability has a stabilizing effect on the coexistence of prairie grasses. *Proceedings of the National Academy of Sciences*. 103: 12793-12798.

Ågren, J. & Willson, M.J. 1994. Cost of seed production in the perennial herbs *Geranium maculatum* and *G. syvaticum*: an experimental field study. *OIKOS*. 70: 35-42

Allan, E. van Ruijven, J. & Crawley, M.J. 2010. Foliar fungal pathogens and grassland biodiversity. *Ecology*. 91: 2572-2582.

Banks, J.E. 2004. Divided culture: integrating agriculture and conservation biology. *Frontiers in Ecology and the Environment*. 2: 537-545.

Benson, E.J. & Hartnett, D.C. 2006. The role of seed and vegetative reproduction in plant recruitment and demography in tallgrass prairie. *Plant Ecology*. 187: 163-177.

Bizecki Robson, D. 2013. An assessment of the potential for pollination facilitation of a rare plant by common plants: *Symphyotichum sericium* (Asteraceae) as a case study. *Botany*. 91: 43-42.

Brooker, R.W. 2006. Plant-plant interactions and environmental change. *New Phytologist*. 171: 271-284.

Burton, G.W. & DeVane, E.H. 1953. Estimating heritability in tall fescue (*Festuca arundinacea*) from replicated clonal material. *Agronomy Journal*. 45: 478-481.

Callaway, R.M. & Aschehoug, E.T. 2000. Invasive plants versus their neighbours: A mechanism for exotic invasion. *Science*. 290: 521-523.

Cane, J.H. 2006. An evaluation of pollination mechanisms for purple prairie-clover, *Dalea purpurea* (Fabaceae: Amorpheae). *American Midland Naturalist*. 156: 193-197, DOI: 10. 1674/0003-031(2006)156[193:AEOPMF]2.0.CO;2.

Casler, M.D. & Brummer, E.C. 2008. Theoretical expected genetic gains for among-and-within-family selection methods in perennial forage crops. *Crop Science*. 48: 890-902.

Cattani, D.J., Elliott, B., Ostermann, D. & Northover, P. 2009. *Summary of the Manitoba forage seed reports for 2009*. (Available at: www.umanitoba.ca/afs/agronomists_conf/2009/Posters/Cattani_poster_forage_seed.pdf).

Cattani, D.J., Miller, P.R. & Smith Jr, S.R. 1996. Relationship of shoot morphology between seedlings and established turf in creeping bentgrass. *Canadian Journal of Plant Science*. 76: 283-289.

Cattani, D.J., Gjuric, R., Feindel, D.E., Miller, P.R. & Smith Jr, S.R. 1997. Management requirements for dryland creeping bentgrass seed production. In *Proceedings of the XVIII International Grassland Congress*. 25: 25-52.

Cattani, D.J., Smith Jr, S.R., Miller, P.R., Feindel, E.E. & Gjuric, R. 2004. Seed yield of creeping bentgrass entries in Manitoba. *Canadian Journal of Plant Science*. 84: 117-124.

Cattani, D.J., Struik, P.C. & Nowak, J.N. 2002. Comparative morphological development of divergent flowering types of annual bluegrass and tillering types of creeping bentgrass. *Crop Science*. 42: 754-762.

Chen, Y.H. & Welter, S.C. 2005. Crop domestication disrupts a native tritrophic interaction associated with the sunflower, *Helianthus annuus* (Asterales: Asteraceae). *Ecological Engineering*. 30: 673-683.

Cheplick, G.P. 1995. Life history trade-offs *in Amphibromus scabrivalvis* (Poaceae): Allocation to clonal growth, storage, and cleistogamous reproduction. *American Journal of Botany*. 82: 621-629.

Clement, S.L., Griswold, T.L., Rust, R.W., Hellier, B.C. & Stout, D.M. 2006. Bee associates of flowering *Astragalus* and *Onobrychis* genebank accessions at a Snake River site in eastern Washington. *Journal of the Kansas Entomological Society*. 79(3): 254-260.

Christie, B.R. & Kalton, R.R. 1960. Recurrent selection for seed weight in bromegrass, *Bromus inermis* Leyss. *Agronomy Journal*. 52: 575-578.

Cox, T.S., Glover, J.R., Van Tassel, D.L., Cox, C.M. & DeHaan, L.R. 2006. Prospects for developing perennial grain crops. *BioScience*. 56: 649-659.

Cox, T.S., Van Tassel, D.L Cox, C.M. & DeHaan, L.R. 2010. Progress in breeding perennial grains. *Crop and Pasture Science*. 61: 513-521.

Crews, T.E. 2005. Perennial crops and endogenous nutrient supplies. *Renewable Agriculture and Food Systems*. 20: 25-37.

CSGA. 2011. *Section 6: Foundation, Registered and Certified Production of Grasses*. (Available at www.seedgrowers.ca/pdfs/Circular%2062010/Circ6-SECTION%2006-ENGLISHRev01-6_20110122.pdf).

DeHaan, L.R., Van Tassel, D.L. & Cox, T.S. 2005. Perennial grain crops: A synthesis of ecology and plant breeding. *Renewable Agriculture and Food Systems*. 20: 5-14.

DeHaan, L.R., Weisberg, S., Tilman, D. & Fornara, D. 2010. Agricultural and biofuel implications of a species diversity experiment with native perennial grassland plants. *Agriculture, Ecosystems and Environment*. 137: 33-38.

Dofing, S.M. & Knight, C.W. 1992. Alternative model for path analysis of small grain yield. *Crop Science*. 32: 487-489.

Dray, S., Pélissier, R., Couteron, P., Fortin, M-J., Legendre, P., Peres-Neto, P.R., Bellier, E., Bivand, R., Blanchet, F.G., De Cáceres, M., Dufour, A-B., Heegaard, H., Jombart, T., Munoz, F., Oksanen, J., Thoulouse, J. & Wagner, H.H. 2012. Community ecology in the age of multivariate multiscale spatial analysis. *Ecological Monographs*. 82: 257-275.

Dyer, L.A., Letourneau, D.K., Chavarria, G.V. & Amoretti, D.S. 2010. Herbivores on a dominant understory shrub increase local plant diversity in rain forest communities. *Ecology*. 91: 3707-3718.

Elgersma, A., Winkelhorst, G.D. & den Nijs, A.P.M. 1994. The relationship between progeny seed yield in drilled plots and maternal spaced-plant traits in perennial ryegrass. *Plant Breeding*. 112: 209-214.

Elgersma, A. & van Wijk, A.J.P. 1997. Breeding for higher seed yields in grasses and forage legumes. *Forage Seed Production: I. Temperate Species*, D.T. Fairey and J.G. Hampton eds. CAB International, UK 1997.

Fletcher, J. 1897. Weeds. Dept. of Agriculture, Central Experimental Farm. *Ottawa Canada Bulletin*. 28: pp.43 and index.

Georgescu, M., Lobell, D.B. & Field, C.B. 2011. Direct climate effects of perennial bioenergy crops in the United States. *Proceedings of the National Academy of Sciences*. 108: 4307-4312.

Gilbert, B. & Levine, J.M. 2013. Plant invasions and extinction debts. *Proceedings of the National Academy of Science*. 110: 1744-1749.

Glover, J.D., Culman, S.W., DuPont, S.T., Broussard, W., Young, L., Mangan, M.E., Mai, J.G., Crews, T.E., DeHaan, L.R., Buckley, D.H., Ferris, H., Turner, R.G., Reynolds, H.L. & Wyse, D.L. 2010. Harvested perennial grasslands provide ecological benchmarks for agricultural sustainability. *Agriculture, Ecosystems and Environment*. 137: 3-12.

Hajjar, R., Jarvis, D.I. & Gemmill-Herren, B. 2008. The utility of cropgenetic diversity in maintaining ecosystem services. *Agriculture, Ecosystems and Environment*. 123: 261-270.

Hayward, M.D. & Vivero, J.L. 1984. Selection for yield in *Lolium perenne*. II. Performance of space plant selections under competitive conditions. *Euphytica*. 33: 787-800.

Heide, O.M. 1994. Control of flowering and reproduction in temperate grasses. *New Phytologist*. 128: 347-362.

Helsen, K., Hermy, N. & Honnay, O. 2012. Trait but not species convergence during plant community assembly in restored semi-natural grasslands. *OIKOS*. 121: 2121-2130.

Hufford, K.M. & Mazer, S.J. 2012. Local adaptation and the effects of grazing on the performance of *Nassella pulchra*: Implications for seed sourcing in restoration. *Restoration Ecology*. 20: 688-695.

Jackson, W. 2002. Natural systems agriculture: a truly radical alternative. *Agriculture, Ecosystems and Environment*. 88: 111-117.

Jonsdottir, G.A. 1991. Tiller demography in seashore populations of *Agrostis stolonifera*, *Festuca rubra* and *Poa irrigata*. *Journal of Vegetation Science*. 2: 89-94.

Kuhnlein, H.V. & N.J. Turner. 1991. *Food and Tradition in History and Anthropology*. Volume 8. Traditional Plant Foods of Canadian Indigenous Peoples: Nutrition, Botany and Use. Solomon H. Katz, Editor. Gordon and Breach Publishers. (Available at www.fao.org/wairdocs/other/ai215e/AI215E05.htm).

Lammerts van Bueren, E.T., Struik, P.C. & Jacobsen, E. 2002. Ecological concepts in organic farming and their consequences for an organic crop ideotype. *Netherlands Journal of Agricultural Science*. 50: 1-26.

Macdonald, H. 1974. *Native Manitoba plants in bog, bush and prairie*. Publication No. 452, Manitoba Department of Agriculture, May 1974.

MacDougall, A.S., McCann, K.S., Gellner, G. & Turkington, R. 2013. Diversity loss with persistent human disturbance increases vulnerability to ecosystem collapse. *Nature*. 494: 86-89.

Ollerton, J., Winfree, R. & Tarrant, S. 2011. How many flowering plants are pollinated by animals? *OIKOS*. 120: 321-326.

O'Mara, F.P. 2012. The role of grasslands in food security and climate change. *Annals of Botany*. 110: 1263-1270.

Pataki, D.E., Carreiro, M.M. Cherrier, J., Grulke, N.E., Jennings, V., Pincetl, S., Pouyat, R.V. Whitlow, T.H. & Zipperer, C. 2011. Coupling biogeochemical cycles in urban environment: ecosystem services, green solutions, and misconceptions. *Frontiers of Ecology and the Environment*. 9: 27-26.

Picasso, V.D., Brummer, E.C., Liebman, M., Dixon, P.M. & Wilsey, B.J. 2008. Crop species diversity affects productivity and weed suppression in perennial polycultures under two management strategies. *Crop Science*. 48: 331-342.

Schaaf, H.M. & Rogler, G.A. 1962. Breeding crested wheatgrass for seed size and yield. *Crop Science*. 2: 347-351.

Smith, R.G., Mortensen, D.A. & Ryan, M.R. 2009. A new hypothesis for the functional role of diversity in mediating resource pools and weed-crop competition in agroecosystems. *Weed Research*. 50: 37-48.

Sperling, L. & Loevinsohn, M.E. 1993. The dynamics of adoption: distribution and mortality of bean varieties among small farmers in Rwanda. *Agricultural Systems*. 41: 441-453.

Stein, C. Unsicker, S.B., Kahmen, A., Wagner, M., Audorff, V., Auge, H., Prati, D. & Weisser, W.W. 2010. Impact of invertebrate herbivory in grasslands depends on plant species diversity. *Ecology*. 91: 1639-1650.

Sturludóttir, E., Brophy, C. Bélanger G., Gustavsson, A.M., Jørgensena, M., Lunnan, T. & Helgadóttir, Á. 2013. Benefits of mixing grasses and legumes for herbage yield and nutritive value in northern Europe and Canada. *Grass and Forage Science*. doi:10.1111/gfs.12037.

Thomas, J.R. & Frakes, R.V. 1967. Clonal and progeny evaluations in two populations of tall fescue (*Festuca arundinacea* Schreb.). *Crop Science*. 7: 55-58.

Tilman, D., Reich, P.B., Knops, J., Wedin, D., Mielke, T. & Lehman, C. 2001. Diversity and productivity in a long-term grassland experiment. *Science*. 294: 843-845.

Tilman, D., Wedin, D. & Knops, J. 1996. Productivity and sustainability influenced by biodiversity in grassland systems. *Nature.* 379: 718-720.

Tulbure, M.G., Wimberly, M.C., Boe, A. & Owens, V.N. 2012. Climatic and genetic controls of yields of switchgrass, a model bioenergy species. *Agriculture, Ecosystems and Environment.* 146: 121-129.

Vellend, M. 2006. The consequences of genetic diversity in competitive communities. *Ecology.* 87: 304-311.

Weinberg, G.M. 1975. *An introduction to general systems thinking*. John Wiley & Sons, New York. 17-19.

Weiner, J., Andersen, S.B., Wille, W.K-M., Griepentrog, H. & Olsen, J.M. 2010. Evolutionary agroecology: the potential for cooperative, high density, weed-suppressing cereals. *Evolutionary Applications.* 3: 473-479.

Wiltshire, K., Delate, K., Wiedenhoeft, M. & Flora, J. 2010. Incorporating native plants into multifunctional prairie pastures for organic cow-calf operations. *Renewable Agriculture and Food Systems*. doi: 10.1017/S174217051000044X 13pp.

Wright, A.J. 1985. Selection for improved yield in inter-specific mixtures or intercrops. *Theoretical and Applied Genetics.* 69: 399-407.

Zhang, D.Y. & Jiang, X.H. 2000. Costly solicitation, timing of offspring conflict, and resource allocation in plants. *Annals of Botany*. 86: 123-131.

Zhao, D.L., Atlin, G.N., Bastiaans, L. & Spiertz, J.H.J. 2006. Cultivar weed-competitiveness in aerobic rice: Heritability, correlated traits and potential for indirect selection in weed –free environments. *Crop Science.* 46: 372-380.

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AGRONOMIC MANAGEMENT OF PERENNIAL WHEAT DERIVATIVES: USING CASE STUDIES FROM AUSTRALIA TO IDENTIFY CHALLENGES

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ABSTRACT

The prospective development of viable perennial cereal crops is as much about developing novel farming systems as it is about developing novel germplasm. Unlike the development of other new crops such as triticale which could be quickly deployed into existing farming systems with only minimal adjustment, a perennial crop will require a substantial re-engineering of the farming system to take advantage of the production and ecosystem service benefits it potentially has to offer. Australia is a country in which pasture and crop production systems rely heavily on exotic species. Farming systems to utilize these species have therefore been developed over many decades and often differ markedly from production systems elsewhere around the world. The objective of this paper is to assess relevant case studies to identify likely challenges in the deployment of perennial cereal crops, with particular reference to perennial wheat. Perennial wheat crops are likely to be dual purpose crops used for grain and forage. They are likely to be for lower input systems that will require appropriate companion species for biological N_2 fixation and possibly to perform pest control functions in a perennial crop polyculture. Adequate nitrogen supply from biological sources will be a key challenge in viable perennial crop systems; the perennial forage grass experience in Australia highlights the chronic nitrogen deficiency that inhibits grass production in a conventional system that relies almost entirely on biologically-fixed sources of N. This paper provides examples of forage species which were developed in Australia that could present as useful templates in the development of a more biological production system based around perennial wheat. It also shows that different countries have different technologies and different perspectives that will potentially add value to the development of novel farming systems. The challenge to develop such novel farming systems will not be met easily and will likely require a multi-disciplinary, multi-institutional and probably global approach.

Keywords: farming systems, low input, monoculture, perennial grains

INTRODUCTION

A recent evaluation of available germplasm has established the feasibility of the concept of perennial wheat (Hayes *et al.* 2012). That study quantified longevity and grain yield for up to three years and demonstrated an association between the capacity to regrow post-harvest and the presence of at least one whole genome equivalent (14 chromosomes) from the perennial donor species. A subsequent study (Larkin and Newell, 2014) has gone on to explore the ramifications of this finding in the context of progressing perennial wheat germplasm development based on the synthesis of complete amphiploids. Larkin and Newell (2014) liken this approach to the development of triticale; the hybridisation of wheat (*Triticum* spp.) and cereal rye (*Secale cereale* L.), and therefore establish that the genetic improvement of perennial wheat germplasm using this approach has a precedent from which knowledge and experience can be drawn.

However, while the genetic improvement of perennial wheat might be able to draw upon the triticale experience, the integration of the novel perennial wheat technology into commercial farming systems has no such precedent upon which to draw. Few perennial cereal crops currently exist in commerce. triticale, once developed, was able to be incorporated into existing annual crop rotations reasonably seamlessly. The availability of perennial cereal crops will present a unique challenge to develop a farming system that is sufficiently flexible to utilize its grain (Cox *et al.* 2010; Hayes *et al.* 2012) and grazing potential (Jaikumar *et al.* 2012; Newell *et al.* 2013), while at the same time allowing the crop to undertake the essential ecosystem services that have ultimately spurred their development in the first place (Glover and Renagold, 2010; Glover *et al.* 2012; Culman *et al.* 2013). Perennial wheat development therefore not only requires refining the genetics of the germplasm but also the development of suitable farming systems.

This review paper uses examples from existing Australian production systems to identify likely similarities and differences between management strategies required for a commercial perennial cereal crop in the future compared with various existing production systems. The emphasis on Australian farming systems is due to: i) similarity in emphasis on 'mixed' livestock and cropping production systems which have been previously identified as key targets for perennial crop technologies (such as in parts of Africa, see Glover *et al.* 2012), ii) Australia's ancient and inherently infertile soils, again a key target for perennial crop technologies, and iii) Australia's long history of development of novel farming systems based on the need to adapt exotic species to agricultural production in a unique and variable landscape/soil/climate matrix (Bell *et al.* 2013). Using examples and with a particular emphasis on perennial wheat, this paper highlights the importance of considering the end use of novel perennial grain technologies to inform priorities in germplasm development programmes and the way in which the germplasm is evaluated along its pathway to market. We assume a perspective of developing perennial wheat for the Australian market, but suggest many examples cited will have a broader relevance beyond the Australian context.

THE AUSTRALIAN CONTEXT

Indigenous Australians were a hunter/gatherer civilization prior to European settlement in 1788. Agricultural production, in the European sense, is therefore relatively new to Australia. European livestock and cropping production systems needed to be adapted to Australian conditions due to the continent's inherently infertile soils (Hubble *et al.* 1983) and one of the most variable rainfall regimes on Earth (Love, 2004). As a result Australia's modern agricultural production systems are based predominantly on exotic species, notwithstanding a small number of exceptions – rangeland production systems being perhaps the most notable (Harrington *et al.* 1984).

Grain cropping in Australia is predominantly carried out in conjunction with grazing and livestock production – so called 'mixed farming' (Bell and Moore, 2012). Although it adds to the complexity of farm business management (Casburn *et al.* 2013) the mixed farming model offers a number of advantages over a single enterprise model. Firstly, a diversified business is more able to manage economic risk associated with inherently variable weather and commodity prices to which Australian farmers are routinely exposed (Hutchings and Nordblom, 2011). Secondly, a diversity of enterprises increases the capacity to utilize different soil types or landscapes that may exist within a given farm (Bell *et al.* 2013). Thirdly, there is the opportunity for synergistic effects between enterprises, such as fixed atmospheric nitrogen from a pasture phase becoming available during a subsequent cropping phase (Dear *et al.* 2004) thus reducing the need for fertilizer inputs. Notwithstanding, external factors such as reduced availability of labour relative to capital (Bell and Moore, 2012) exert significant pressure on Australian farmers to specialize, similar to their counterparts in other developed nations (Russelle *et al.* 2007; Wilkins, 2008).

The importance of pasture legumes to agricultural production on the infertile soils of Australia has been long recognised (Donald, 1965) and well described (e. g. Angus and Peoples, 2012; Peoples *et al.* 2012). However, very few herbaceous legumes native to Australia exist and many of those are toxic to livestock and unsuited to conventional production systems (Cocks, 2001). As a consequence and particularly post Second World War, Australian agriculture has embraced legume development perhaps more than any other country (Nichols *et al.* 2012) with programmes that have culminated in the release of many novel legume cultivars (Nichols *et al.* 2007). Nicholls *et al.* (2012) identify 30 different legume species that are all exotic to Australia but were first commercialised in Australia. Biological N_2 fixation from pasture legumes was the primary source of N for cereal crops prior to 1990 when the broadscale use of synthetic fertilizer N accelerated (Angus, 2001). Despite this management change, biological N_2 fixation remains important to contemporary Australian grain production systems (Angus and Peoples, 2012).

THE TRITICALE EXPERIENCE

Triticale (*Triticosecale* Wittm.) provides a model for the development of a viable cereal crop through wide hybridisation. This wheat × rye hybrid is the most successful synthetic crop species produced (Ammar *et al.* 2004). Compared with wheat, triticale demonstrates superior adaptation to acid soils, drought, cold, infertile soils and has improved disease resistance (Giunta *et al.* 2003; Erekul and Kohn, 2006; Motzo *et al.* 2011). The intergeneric hybrid between hexaploid wheat (*T. aestivum*) and rye (2n =14 = RR genome) produces octoploid triticale (2n =56 = AABBDDRR genome), while using tetraploid wheat (*T. durum*) as a parent produces hexaploid triticale (2n = 42= AABBRR genome). Although the aim of hybridisation in this case was not for perenniality, the same principles can be applied to develop perennial cereals (Larkin and Newell, 2014).

Since the synthesis of triticale, most breeding efforts and improvement programmes have focused on hexaploid types which dominate world utilisation, mainly in animal feeding, both as a forage and grain (Ammar *et al.* 2004). Triticale production in Australia is approximately 0.75 million tonnes annually (J. E. Roake, 2013, personal communication). By comparison the average wheat production is 25 (USDA, 2013), making triticale only a minor component of cereal grain production in this country. triticale fits seamlessly into current cropping rotations, as basic agronomic practices such as seeding, fertiliser management, pest control and harvesting are similar to other cereals. As a dual purpose cereal, triticale offers an alternative to other grains in mixed farming enterprises. The nutritional characteristics of triticale are superior to wheat in terms of amino acids (particularly lysine, Mergoum *et al.* 2004) which makes it a sought after grain in ruminant and monogastric animal industries. However, the higher ash content, lower milling yields and inferior loaf volume and texture are detrimental for use in commercial baking (Salehi and Arzani, 2013). Many of the gene loci responsible for bread making quality (glutenin-encoding genes) are located on the 1D chromosome, which is lacking in hexaploid triticale. However, techniques exist which can identify greater proportions of glutenin content in hexaploid triticale, which would allow selection for improved bread making quality (Salmanowicz and Dylewicz, 2007). A high proportion of alpha-amalayse activity is also common in triticale grain which further limits dough quality and predisposes triticale to preharvest sprouting (Martinek *et al.* 2008). Addressing these grain quality issues will be important for improving market access of triticale into the future. So too, end use capability and market access will be important attributes for the success of perennial cereal crops. Some attention must be given to grain quality and target area of adaptation when developing perennial cereals to prevent these crops becoming “just another” feed grain or forage species.

THE GRAZING CROP EXPERIENCE

Dual purpose crops (wheat, oats, barley, triticale and more recently canola) to produce both forage and grain, have been an integral part of mixed farming enterprises for many years, both in Australia and elsewhere (Dann *et al.* 1983; Virgona *et al.* 2006; Kirkegaard *et al.* 2012; Tian *et al.* 2012). The ability of annual crops to produce large quantities of herbage during autumn and winter offers an opportunity to rest pastures during this key period (McMullen and Virgona, 2009). The ability to graze dual purpose crops and produce harvestable grain, also improves the gross margin of the farming system and acts as insurance against harvest failure in a poor season and fluctuating commodity prices. This enables increased flexibility in decision making for the farm manager (Virgona *et al.* 2006; Moore, 2009).

Production from dual purpose crops requires a higher level of management as it requires earlier autumn sowing and then grazing in the winter. Earlier sowing takes advantage of warmer autumn temperatures for better crop establishment and crop vigour (Harrison *et al.* 2011). However, earlier sowing can predispose cereal crops to disease because of increased activity of fungal pathogens in warmer temperatures (Virgona *et al.* 2006). Effective break crops and resistant varieties are important to combat these issues, as are the use of pesticides. Similar issues are faced with canola as a grazing crop and the use of resistant varieties and careful grazing management are required to reduce disease incidence (Kirkegaard *et al.* 2012). While spring type cultivars can be used as dual purpose crops, winter types are favoured because of their longer period of vegetative growth. Grazing needs to be managed so that animals are removed before stem elongation. Once the crop matures to the reproductive stage, apical meristems rise quickly with stem elongation, increasing the possibility of removal by grazing and subsequent reduction in grain yield. Inputs of nitrogen (N) fertiliser are required following grazing to replace N removal by animals and to improve grain protein, especially in cultivars with higher grain quality (Virgona *et al.* 2006). While there are many factors that influence grain yield in dual purpose crops, grazing generally reduces grain yield (Harrison *et al.* 2011). However, with precise management grazing can have a positive effect on grain yield of grazed crops compared with their ungrazed counterparts. Grazing lengthens development and delays water use in crops (Virgona *et al.* 2006; McMullen and Virgona, 2009). This water can be conserved and used more effectively after anthesis, when assimilation is directed toward grain yield, thereby increasing water use efficiency. Earlier sowing also leads to deeper roots, increasing access to moisture in the soil profile. The delayed development can allow crops to respond to late season rain in favourable seasons and greater water use efficiency can improve yields under drier conditions.

Cereal forages are known to have extremely high nutritive value and to support high growth rates in sheep (Moore, 2009). However there is concern that the nutrient content of these forages may limit growth rates of grazing animals and lead to nutritive disorders (Berger, 1992; Dove and McMullen, 2009), particularly regarding the ratios of potassium (K), magnesium (Mg), sodium (Na) and calcium (Ca). All cereals contain sufficient Ca for ruminant dietary requirements but K contents can be up to ten times the required intake, while the Mg and Na content for wheat in particular, is generally below dietary requirements (Dove, 2007). Winter grazing of grass monocultures that have high levels of protein and K with relatively low quantities of Mg and Na causes reduced Mg adsorption in the rumen. This inhibits weight gain in animals and can lead to the hypomagnesaemia (grass tetany) disorder (Brightling, 1994). Mineral Supplements are required to correct these deficiencies. Indices of cation ratios can be helpful in deciding the supplement requirements from different forages. Cation ratios K/(Mg + Ca) exceeding 2.2 indicate the need to supplement diets with Mg. Cation ratios for wheat have been reported as high as 3.7 (Dove and McMullen, 2009). By comparison cation ratios for subterranean clover (*Trifolium subterraneum* L.) range from 0.9-1.3 (Dove, 2007). Indices for K:Na have not been quantified, however Na supplementation is also recommended when grazing winter wheats (Dove, 2007). These findings suggest that adverse animal health implications of grazing a perennial cereal crop could be reduced where the crop is grown as part of a polyculture in which grazing animals are exposed to different forages.

THE PERENNIAL FORAGE GRASS EXPERIENCE

Associated with Australia's extensive breeding, development and commercialisation of annual forage legumes from the Mediterranean Basin was the early realisation that these legumes had to be grown in a mixture with a productive companion species. This was necessary because pure annual legume swards tend to be prone to weed invasion and thus not very productive. Growing these legumes with a grass companion greatly increased overall productivity and because the N fixed by the legume would be used by the grass companion the botanical stability of the sward was also improved. The most common grass companion during the early stages of development of this pasture technology was annual ryegrass (*Lolium rigidum*; Oram, 1990). This species is rarely recommended now because of several disadvantages including (a) weed potential to cereal crops, (b) alternative host of several cereal diseases and (c) the annual habit may exacerbate soil problems including erosion, waterlogging and acidification. Perennial pasture grasses, such as phalaris (*Phalaris aquatica*), cocksfoot (*Dactylis glomerata*) and tall fescue (*Festuca arundinacea*) are broadly utilised in Australian farming systems because they do not have the disadvantages of ryegrass or other annual grasses and have been shown to enhance farming system sustainability (Dear *et al.* 2007).

The temperate perennial grasses that are important in the mixed farming zone of southern Australia, phalaris, cocksfoot and tall fescue all contain a wide degree of genetic variability associated with the fact that all their zones of origin encompass large tracts spreading from Eurasia, across the Mediterranean Basin to the verges of the Sahara in North Africa (Anderson, 1961; Borrill, 1972; Lumaret, 1988). Indeed, a key element of the development of appropriate adaptation in these grasses for Australia has involved germplasm discovery in Eurasian and north African isoclimes (Neal-Smith, 1955). Because the zones of adaptation of these species cover such large regions, the range of climates to which adaptation within any one of these species is found is also large. Thus populations from the cool to cold temperate, summer rainfall dominant zones in north-western Europe typically are summer active while those from arid Mediterranean climates of North Africa with summer dry periods of four months or more are winter active and summer dormant (Cooper, 1963). Indeed, some of the grass cultivars best adapted to Australia's drier mixed farming zones, e.g. Sirocco phalaris, Kasbah cocksfoot and Resolute tall fescue, trace their parentage back to populations collected during expeditions to Africa and the Near East. Ideally, perennial donor species for perennial cereal crops intended for Australian environments would also be sourced from Eurasian and/or north African environments, if no suitable native species could be identified.

Perennial grasses able to survive the hot, dry summers which are typical of southern Australia generally have to express one or a combination of traits including summer dormancy (Volaire and Norton, 2006), dehydration avoidance (Norton *et al.* 2012), or dehydration tolerance (Volaire and Conejero, 2001) to ensure survival. In addition lenient grazing over the summer may also be required because the joint stresses imposed by defoliation and drought, which threaten both plant carbohydrate reserves and water status, are important in reducing survival of perennial pasture grasses (Volaire, 1995; Hacker *et al.* 2006). In addition, some species, e.g. cocksfoot, typically shed many roots over dry summers and this may make them susceptible to being pulled out of the soil by livestock if grazing occurs before replacement roots have regrown sufficiently (Ridley and Simpson, 1994). It will be important to understand the nature of the perennial cereal crop rooting structure in order to develop a grazing system that maximises productivity but does not compromise plant persistence.

The length of time that a sown perennial grass based pasture is likely to persist is a key determining factor that the farmer must consider when deciding whether or not it is economic to sow a new pasture. The decision will also be influenced by the costs associated with pasture improvement and the extra income that the farmer will likely obtain from the improvement. Decision support tools are increasingly being used to assist in the making of these decisions (e.g. www.evergraze.com.au/tools.htm). However, in any case there will be a minimum amount of time over which the newly sown pasture must persist and produce for the farmer to recoup his investment (break-even period) and this will be influenced by the genetic makeup of the new pasture (i.e. how well it is intrinsically adapted to the environment), and the management that the pasture experiences. Commonly break-even periods of 6-10 years are quoted for pasture resowing although depending upon the rate of return required on investment this may even increase to 20 years (Scott *et al.* 2000). As a consequence persistence is acknowledged as an important attribute that any successful cultivar should possess. Longevity of a perennial cereal crop will too be an important factor in determining its economic viability, though income received from grain yields would likely reduce its required persistence, compared with a perennial forage grass.

When the agronomic practises used for the various types of grasses are considered, the perennial grass crop which is maintained primarily for seed production is the closest analogue we have which mimics a perennial, dual-purpose, forage/grain cereal. The management of any perennial grass will, by necessity be quite different depending upon whether it will be used solely for forage or have a dual purpose as a forage and grain/seed crop. The practise absolutely essential to maximise grain/seed yield is to protect the reproductive tillers from defoliation. This necessitates the removal of grazing animals or the cessation of cutting prior to when reproductive tiller elongation commences. Similarly management practises, e.g. sward renovation, fertilisation, plant protection etc. to maximise the density of fertile reproductive tillers which are initiated will optimise yield potential. In the same way reducing the likelihood of lodging, perhaps by application of straw shortening hormone, during the late reproductive growth stage is increasingly recommended to improve seed yield recovery.

In contrast, for a grass whose primary function is forage production the protection of the reproductive tillers is generally not particularly essential. This is especially so because for most perennial forage grasses recruitment of young plants from seed is not the primary means of sward perenniation but rather the long term survival of adult plants. Perennial grasses grown for forage in Australia are almost always sown with a companion legume the aim being two-fold, to improve sward forage quality and enhance soil nitrogen status through biological N_2 fixation. In contrast, perennial grass seed crops are rarely grown with a companion legume as the seed is usually of high value so that seed producers are able to absorb fertilisation costs. Moreover, the agronomy required for maintaining a mixed grass/legume sward is more complicated than pure grass culture and the favourable returns make additional complexities unnecessary. In contrast, the value of grain produced by a perennial cereal will certainly be much less than seed produced by a perennial forage grass. Consequently the forage produced by the perennial cereal will assume an important part of the overall value of the crop, particularly if as demonstrated in a previous modelling exercise, it is produced, ‘out-of-season’ in autumn in southern Australia (Bell *et al.* 2008). Whether substantial ‘out-of-season’ autumn forage production will be achievable from a cool season perennial grass at a time of the year normally quite dry remains to be seen.

MONOCULTURES VERSUS POLYCULTURES

Various authors have suggested that a move toward perennial crops may also represent a move away from the reliance on monocultures (Cox *et al.* 2006; Glover *et al.* 2007; Glover and Renagold, 2010; Glover *et al.* 2012). Recent research in forage species would support the notion that perennial crop-based polycultures could be commercially feasible to the extent that increased species richness on average increases total biomass productivity and weed suppression in perennial herbaceous polycultures (Tracey and Sanderson, 2004; Picasso *et al.* 2008; 2011). However, this is in a pure forage production system – what negative impacts could we anticipate on grain production of perennial cereals grown in a mixed sward?

The fundamental basis for targeting a polyculture system is to enhance resource utilisation through complementarity of companion species (Glover and Renagold, 2010; Picasso *et al.* 2011). The most obvious example of complementarity is the synergy between a N_2-fixing legume providing N to non-legume species growing in the same sward. In designing perennial crop-based polycultures, it would seem the integration of appropriate companion legumes would be an obvious place to start. Nitrogen is a critically important macro-nutrient for forage and grain-crop species alike, and a farming system that reduced or eliminated the need for synthetic nitrogenous fertiliser would quickly achieve many of the fundamental imperatives of a perennial crop, such as reduced nutrient leakage and lower input requirement (Glover *et al.* 2007, 2012; Glover and Renagold, 2010). Yearly nitrogenous fertiliser consumption for annual cereals grown in Australia totals 702×10^6 kg/ha, the highest use of any agricultural enterprise (Chen *et al.* 2008). A great proportion of the applied nitrogen is lost, with efficiencies of uptake for cereals such as wheat quoted at 41 percent (Chen *et al.* 2008). The loss of nitrogen represents a significant business inefficiency for farmers as well as having negative implications for the environment and human health. Perennial grains grown in polyculture could provide a way of reducing synthetic nitrogenous fertiliser use in cereal grain production systems.

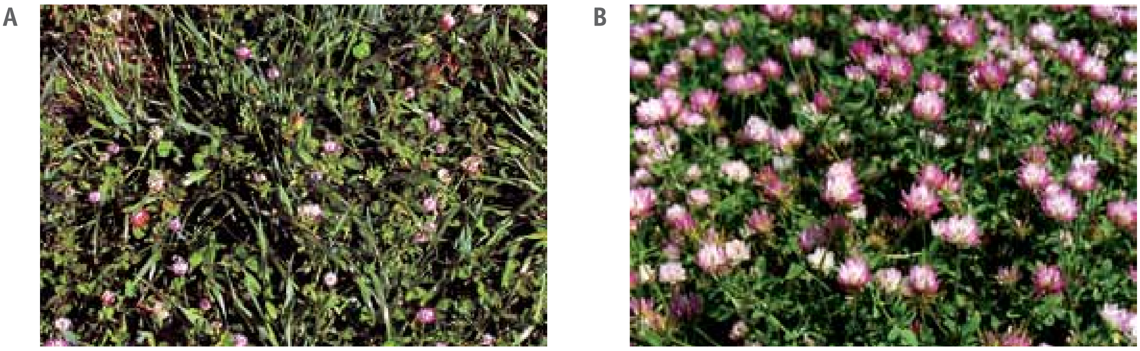
Several surveys conducted across southern Australia (e.g. Fortune *et al.* 1995; Bowman *et al.* 2004; King *et al.* 2006) have commonly found legume composition in commercial mixed pasture swards to be inadequate. The reasons for this are varied but it highlights a practical challenge which is likely to exist if we also expect legumes to coexist with perennial cereal crops. One contributing factor to the Australian experience is the paucity of adapted perennial legume species, particularly in drier cropping-zone environments (Cocks, 2001; Dear *et al.* 2003a; Li *et al.* 2008). Therefore Australian pasture swards are overwhelmingly reliant upon annual legumes such as subterranean clover (*Trifolium subterraneum*) and barrel medic (*Medicago truncatula*). Perennial crops in Australia will also probably be reliant upon annual legume species unless the target environment is the high rainfall permanent pasture zone where white clover (*T. repens*) and Caucasion clover (*T. ambiguum*) are more likely to be adapted (Virgona and Dear, 1996; Lane *et al.* 2000), though seed of the latter species is difficult to obtain at present. Mixtures with alfalfa

(*Medicago sativa*) is a possibility (Boschma *et al.* 2010) in the cropping zone although its supreme capacity to extract soil water in moisture limiting environments (Hayes *et al.* 2010b) is likely to have a negative effect on companion perennial crops similar to its effect on companion annual legumes (Dear and Cocks, 1997) or over-sown cereal cover-crops (Norton and Koetz, 2013).

The nitrogen requirement of perennial cereal crops needs to be determined so that the 'adequate' legume content can be defined. Perennial forage grasses are highly responsive to N fertiliser (e.g. Mills *et al.* 2006) although critical N requirements of common perennial grass forages in Australia are still to be defined. Stork and Jerie (2003) calculated the relative uptake of inorganic N between late autumn and early spring (1 year only) to be 169 kg N ha^{-1} under one year old phalaris. However, Dear *et al.* (1999) demonstrated that a phalaris/subterranean clover mixed sward was only capable of fixing 143-177 kg N ha^{-1} over three years; less than one third the requirement of the perennial grass component, even ignoring the fact that only a proportion of total N fixed in a mixed sward will become available to the grass. Therefore in an Australian system we could expect that perennial grasses grown in mixed commercial swards would typically exist in an almost permanent state of N deficiency. The N-status for perennial cereal crops could be even more constrained due to the elevated N demand for grain production. This needs to be quantified, as does the importance of timing of N supply to a dual purpose cereal crop. The N status of a mixed perennial grass pasture sward in Australia is likely to be better in autumn due to increased mineralisation and reduced N demand over summer, while supply in spring is likely to be much more limiting and it is unclear what implications this would have on grain yield, grain quality and longevity of a perennial cereal crop.

Nitrogen nutrition is more complex than other nutrients because of the strong relationship between plant growth, nitrogen availability and available soil water.

FIGURE 1. PERENNIAL GRASS AND ANNUAL FORAGE LEGUME



A. **A mixed forage pasture sward containing a perennial grass (*Phalaris aquatica*) and self-regenerating annual legume species (*Trifolium subterraneum*, *T. michelianum* and *T. glanduliferum*)**
B. **Gland clover (*T. glanduliferum*); A self-regenerating annual forage legume released commercially in Australia for its superior insect pest resistance**

Assumptions can be made to calculate the N requirement of conventional wheat. Following Glendinning (2000); if we assume a grain protein content of 11 percent and a protein conversion factor (PCF) of 1.75[1], the estimated grain nitrogen yield is 11×1.75 = 19.2 kg N/tonne grain. The quantity of soil nitrate required is a function of grain nitrogen yield divided by the N-uptake efficiency (NUE). NUE (efficency with which fertiliser N is converted to grain protein) varies according to the physiological state of the plant, but is estimated to range from 50 percent around sowing down to approximately 15 percent at head emergence (White and Edwards, 2008). Assuming a 50 percent NUE, the estimated quantity of soil nitrate required to produce 1 tonne of grain is 19.2/0.5 = 38.5 kg N/tonne. Therefore an average 3 tonnes/ha wheat crop would require 115.5 kg of nitrate N. However, as the grain protein content increases, the NUE decreases, as the crop has difficulty accessing enough water to use the extra nitrogen required for increased protein (Herridge, 2011). Thus at a grain protein level of 14 percent, NUE falls to 34 percent, requiring 75 kg N to produce a tonne of grain.

Assuming 25 kg of atmospheric N is fixed in annual legume shoots for every tonne of DM of legume shoot biomass produced (Dear *et al.* 1999) and making an allowance for an additional 20 kg fixed N/tonne legume DM associated with or derived from legume roots (Peoples *et al.* 2012), total N fixed biologically is equivalent to 45 kg/tonne of total legume DM. Assuming that 50 percent of the total N fixed becomes available to the crop, a wheat crop yielding 3 tonnes/ha at 11 percent protein would require 5 tonnes/ha of legume biomass to supply nitrogen to the system. A number of limitations are acknowledged with the above calculations:

1. the N requirement to produce 1 tonne of grain from perennial wheat may differ to that estimated for annual wheat due to factors such as a different PCF or different grain protein level
2. the proportion of legume N derived from atmospheric N_2 will vary according to legume species, seasonal conditions and soil factors
3. the actual proportion of legume N available to the companion crop will be dependent upon factors such as grazing strategy, mineralisation rates and transfer mechanisms between crop and legume plants.
4. in a dual purpose crop, an allowance needs to be made for N removed due to grazing

Nevertheless, the above provides a starting point in determining how much N a perennial wheat polyculture might need, and illustrates the high legume content required if biologically fixed N would be the sole source of N. To put these values into context, total pasture herbage production of various perennial-based pasture swards in two representative Australian field environments ranged from 24-31 tonnes/ha over five years (Hayes *et al.* 2010a) or an average of 5-6 tonnes/ha/year. In such an environment where biomass production can be so low, a major question must be whether a perennial crop grown with the robust companion legume component

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[1] The PCF for other crops is commonly 1.6; the PCF for perennial wheat is unknown and will need to be determined

necessary to supply the crop's full N requirement can be commercially viable? It is possible that a system that uses legumes in addition to strategic applications of N fertiliser be developed to reduce the legume composition necessary to supply adequate N, although Peoples *et al.* (2012) highlight the negative effect N fertiliser can have on biological N_2 fixation.

A polyculture is clearly an advantage relative to a monoculture from the perspective of grazing due to: i) the reduced likelihood of adverse animal health conditions and ii) improved forage quality. Grazing ruminants have evolved to ingest a diverse diet, and significant health disorders can ensue if that diversity is not maintained. In general, the ruminant gut relies on an ecosystem of microorganisms to break down food, and the composition of that ecosystem is dynamic and responsive to a changing feed source (Cottle, 1991). Where an imbalance occurs in the diet, there is risk of an imbalance in the gut which can lead to negative effects on health that are sometimes irreversible. These negative effects can occur even when the diet is of high quality, for example, red gut (Gumbrell, 1997) and bloat (FitzGerald *et al.* 1980) from legume pastures or acidosis and lupinosis from grain diets (Brightling, 1994), highlighting the risks to animal health that high quality but imbalanced diets can present to ruminant livestock. The grass tetany example mentioned earlier relating to grazing annual cereal crops is perhaps more indicative of the type of disorder a monoculture perennial cereal crop may present. Regardless, these disorders can usually be managed if there is a balanced diet highlighting a clear advantage of a dual purpose crop grown in a polyculture as opposed to a monoculture.

In addition, a vibrant legume component can improve forage quality for the grazing livestock. Legumes, such as alfalfa, often have a higher protein content and concentration of minerals in their shoots than grasses (e.g. Hayes *et al.* 2008; Hayes *et al.* 2010a). Thus, their presence in a mixed legume/grass sward presents as a high quality component available to browsing livestock. Moreover, the presence of a legume can significantly enhance the quality of the grass herbage through increased N-supply to the grass. Mills *et al.* (2006) showed that the addition of N increased the crude protein of cocksfoot herbage by up to 4-fold and more than doubled the metabolisable energy, in part a reflection of the doubling of pasture growth rates due to additional N. The ability of legumes to substantially enhance the N supply to companion grasses primarily relies on the pasture sward containing a substantial legume content (Peoples *et al.* 2012) and depends upon the efficiency of transfer of N between the legume and grass components.

In its simplest form, a perennial cereal/forage legume polyculture is only a binary mixture and it is acknowledged that production benefits of mixtures may not be fully realized until three or more complimentary species are included (Tracey and Sanderson, 2004). As described above, nitrogen fixation from a legume component is an obvious and easily defined benefit of a polyculture but there are potentially other benefits. Glover *et al.* (2012) describe the 'push-pull' benefits alternative species may offer in controlling pests, particularly in low input production systems. Very briefly the 'push' refers to species that can repel pests thus pushing them away from the valuable crop plant; the 'pull' referring to plants that can attract pests towards them and away from the crop plant. The effectiveness of a push-pull strategy is likely to be site dependant and will vary according to factors such as the type and severity of the pest incursion and the availability of plants with properties capable of pulling or pushing the pest(s) in question. However, there would appear to be enormous potential for research as the push-pull potential of most species is poorly understood.

There are undoubtedly candidates from Australia's various plant development programmes that may have a use in a push-pull context despite this not being the primary motivation for their development. Gland clover (*T. glanduliferum*) was commercialised by Australian scientists for use as a novel alternative in the pastures of mixed farming systems in medium rainfall environments (Nichols *et al.* 2007), particularly on heavier soils (Dear *et al.* 2003b). It has a unique resistance to various insect pests such as redlegged Earth mite (*Halotydeus destructor*), as well as blue green (*Acyrthosiphon kondoi*) and cowpea (*Aphis craccivora*) aphids and although it has been evaluated in forage mixtures (Dear *et al.* 2002; Hayes *et al.* 2008), its potential as a 'push' species was never specifically tested. Biserrula (*Biserrula pelecinus*) is another novel annual legume species also released by Australian scientists as a viable small-seeded legume for pasture crop rotations, particularly where acid soils constrain production (Nichols *et al.* 2007). Sheep tend to avoid grazing this species at certain periods during the year which has fostered its promotion as part of a non-herbicide weed control strategy. It is possible that this species could be incorporated in a non-herbicide weed control perennial crop based system.

These examples highlight two potential opportunities for future perennial grains research. Firstly, they demonstrate that alternative species already exist in commerce around the globe that may have potential to provide complimentary benefits to perennial crops but are yet to be tested in this context. Secondly, it reminds us that germplasm may have existed in previous plant development programmes with similar potential but which were never commercialised because the target market at the time was not focussed on complimentary benefits to other species (certainly not perennial crops) but instead the potential contribution these species could make to production systems in their own right. In a perennial grains context it will be important to define the essential characteristics required of companion species to guide the selection of companion species with which early generation perennial crops should be tested. Both examples above are legume species capable of high rates of biological N_2 fixation, highlighting the likelihood that companion species in a perennial crop context will be required to perform more than one function.

GERMPLASM AND FARMING SYSTEM CO-DEVELOPMENT

The vision for diverse perennial grain cropping systems to replace monocultures of annual crops has been described by various authors previously (e. g. Glover *et al.* 2007; Cox *et al.* 2010). However, in contrast to Cox *et al.* (2010) who suggested that "before such systems can be deployed and tested, new perennial...crops must be developed through breeding", we contend that the process of crop development and farming system engineering should not be separated.

Moreover, the perennial grain crop will ultimately be more successful in commerce and achieve greater environmental impact if the development of both the crop and the farming system occurs concurrently, and better still, if one informs the other in an iterative, multi-disciplinary process.

The temperate perennial forage grass experience in cropping environments of southern Australia provides a useful example highlighting the importance of co-development of the germplasm and the grazing system. Phalaris – another example of an exotic forage species that was primarily developed in Australia (Oram *et al.* 2009) – and cocksfoot are key temperate perennial grass forages in Australia. However, though they are used widely in higher rainfall, permanent pastures, only a few cultivars of either species exist that are suited to drier cropping environments and seed of these cultivars is incredibly difficult to obtain - Australia is currently experiencing market failure in regard to these cultivars. We contend that a major cause of the current market failure was the failure of the local industry to develop the farming system adequately. There is little doubt that the cultivars, primarily Sirolan phalaris (Oram *et al.* 2009) and Kasbah cocksfoot (Oram, 1990), are agronomically suitable to their target environment (Hackney *et al.* 2006 and unpublished data). However, their addition to the phased farming system of southern Australia brought additional complexity to the management regimes which was never adequately explored by the research community and probably explains much of the reason why farmers felt it easier to leave these species out of their rotations. For example, no selective grass herbicides currently exist which are registered for use to control annual grass weeds in establishing perennial grass-based swards (Dear *et al.* 2006) and no integrated weed management strategy has been developed or tested to provide farmers with workable strategies to control their grass weeds in a perennial-grass based sward. Likewise, no previous research has tackled the nitrogen question in relation to perennial grass swards. Farmers require their pasture phase to leave adequate residual soil N for use by subsequent crops (Angus, 2001). Perennial grasses are known to be highly competitive with annual legumes and therefore suppress nitrogen fixation of the total sward (Dear and Cocks, 1997; Dear *et al.* 1999; Dear *et al.* 2000). But no research has yet answered the question of how perennial grasses can be included into cropping rotations in such a way that maintains adequate levels of biological N_2 fixation. Due to the lingering questions about management issues such as weed control and N supply, Australian farmers in the mixed farming zone have avoided utilising these grass cultivars, which has sent feedback through the seed supply chain over the last two decades rendering these cultivars commercially unviable and culminating in a situation in which even a progressive farmer would find incorporating these species problematic due to the paucity of commercial seed.

An existing research project, EverCrop™ (Llewellyn *et al.* 2013), is currently examining the problems around the inclusion of perennial grasses into cropping systems and is trialling the practice of planting mixed swards in monoculture rows. However, an additional problem facing this project is the negative perceptions that some farmers have of perennial grasses in these systems on account of their previous bad experience with the technology. Because the cultivars were developed in isolation from agronomy management research, they failed to meet the expectations of farmers. On account of prejudices which exist with some farmers in relation to these grasses, it is now much more difficult for researchers to achieve meaningful practice change on farm. It is our contention that perennial wheat developers should heed the lessons of Australia's perennial grass experience; perennial wheat technology is likely to be much more successful if a suitable farming system exists at the time the germplasm first becomes commercially available, and the integration and adoption of the new technology will be easier to achieve if bad commercial experiences are avoided.

REFLECTING UPON INITIAL EVALUATION OF PERENNIAL CEREALS

The initial evaluation of perennial wheat derivatives undertaken in Australia (Hayes *et al.* 2012) established the feasibility of the concept of perennial wheat and helped researchers define a strategy for continued development of the wheat × wheatgrass germplasm (Larkin and Newell, 2014). Despite the constraints of limited seed supply hampering the evaluation, a number of inferences could be drawn from the initial study with regard to the likely farming system required of a perennial wheat crop in Australia. It was established that early-generation perennial wheat derivatives were unlikely to persist unless they were grown in high-rainfall environments. Without the introduction of summer dormancy traits the germplasm is unlikely to be able to persist through the hot and dry summer conditions typical in most conventional Australian cropping environments. This is certainly the experience of temperate perennial forage grasses in Australia (Hackney *et al.* 2006; Hayes *et al.* 2010a) and elsewhere (Malinowski *et al.* 2005; Norton *et al.* 2006a,b). However, as with perennial forage grasses it is acknowledged that zones of adaptation can change with continued plant breeding and development. This insight on zone of adaptation immediately provides direction as to the initial companion species that might be targeted in an Australian context; annual and perennial legume species suited to higher rainfall/permanent pasture regions.

However, the initial evaluation was conducted on monoculture, and in many cases, on single row plantings, neither of which is likely to be relevant to commercial perennial wheat plantings. We acknowledge that there will always be a role for testing germplasm in monocultures and, as with the initial evaluation in Australia, monoculture testing is sometimes unavoidable. But if perennial wheat is ultimately envisaged to be grown in polycultures, we suggest that evaluations of perennial crop technology in polyculture occurs early in the development pathway to ensure that the technology is relevant to the situation for which it is intended and that when a perennial crop technology becomes commercially available, a good body of knowledge already exists as to the appropriate management strategies. Most evaluations of forage species in Australia, both legumes and grasses, assess species and cultivars in monocultures. It is understandable why this occurs in the context of measuring relative performance and eliminating all possible sources of error. However, for species that are never to be used as monocultures commercially, surely it is a failing never to test them in their commercial setting until after release. The case for evaluating perennial grains in polycultures is even stronger than for forage species in Australia because not only does the evaluation process screen germplasm, it also develops and refines management strategies for this novel technology.

One final observation: the initial evaluation of perennial wheat in Australia (Hayes *et al.* 2012) was unexpectedly successful. The project had a relatively small budget, none of the germplasm tested was developed for the Australian environment, and none of the research team had previous experience in growing perennial cereal crops. However, the project was able to establish the biological feasibility of perennial wheat crops in Australian environments and could associate the capacity for PHR with the addition of one extra genome equivalent from the perennial donor, thus paving the way for the development of a breeding strategy for the crop (Larkin and Newell, 2014). The success of the research initiative was in no small way attributable to the collaborative, multi-disciplinary approach the project took. The collaboration first relied upon an institution, the Future Farm Industries Cooperative Research Centre, to invest in genuinely '*blue-sky*' research and on research providers (NSW DPI, CSIRO and Charles Sturt University) to co-invest with infrastructure and the valuable time of their staff. Second, it relied upon generous contributions by partner agencies on the other side of the globe, The Land Institute and Washington State University, to provide free and ready access to their best available germplasm. Third, it relied upon the competency of a research team which collectively possessed a broad range of skills including crop agronomy, pasture agronomy and adaptation, physiology, genetics, molecular biology and cereal chemistry. The Australian team also had an advantage in that they were not constrained to biological methods for conducting experiments. This meant they were free to use herbicides, fungicides and fertilisers as appropriate which no doubt assisted in evaluating the genetic potential of the germplasm. It is likely that future success in the challenging field of perennial grain development will also require a multi-disciplinary, multi-institutional and probably international approach.

SUMMARY AND CONCLUSION

Triticale, the most successful hybrid crop, establishes a precedent by which genetic improvement in perennial wheat might be achieved. Despite its superior adaptation to a range of biotic stresses triticale is underutilised in Australia due to its inferior grain qualities, particularly properties important to commerce viz. baking and malting, rendering its grain less valuable than other cereals such as wheat and barley. This is a reminder that the end use of the product is an important consideration determining the extent to which benefits of the genetic gain achieved in the breeding of this crop are realized in the commercial world. So too, the end use of perennial crops needs to be defined and articulated early in the development process to facilitate maximum impact of the technology.

Existing grazing crops provide confidence that the dual-purpose attributes of a perennial cereal will likely add to the flexibility and resilience of mixed farming enterprises, capitalising on the financial benefits associated with diversified income streams as well as improved adaptation to variable weather patterns. Avoiding animal health risks associated with grazing monocultures, and meeting the elevated N requirement caused by N-removal from grazing livestock will be key challenges to be met in perennial cereal production systems. However, the use of perennial cereals in polycultures with legumes will potentially overcome both these constraints.

The temperate perennial forage grass experience in southern Australia provides both hope and caution for the development of viable perennial cereal production systems based on polycultures. Perennial forage grasses in Australia typically rely almost entirely on biological N_2 fixation from pasture legumes and thereby present as an example of a viable polyculture production system. However, a range of factors such as the paucity of legume species available (particularly perennials), the variability of N_2 fixation in the field and the competition between the grass and legumes growing in the same sward mean that perennial grasses likely exist in an almost permanent state of N-deficiency. Using figures from existing industries, early indications suggest that the N-requirements of perennial cereal crops would be even greater than that for perennial forage grasses due to the need to maximise grain yield and grain quality. The suggestion offered in this paper of 5 tonnes of legume DM required in a polyculture to supply adequate N for 3 tonnes of perennial wheat grain could be proven incorrect in time if our various assumptions are wrong, but it paints an ambitious picture of the potential composition of a perennial wheat/legume polyculture raising questions as to whether a polyculture that supplied 100 percent of the crop's N requirement is commercially feasible. This is a priority area of research in perennial crop development. If a polyculture remains the preferred model for perennial cropping systems, the multiple roles of the companion species need to be defined so that suitable species are identified and tested. This paper highlights the potential of legumes commercialised in Australia to perform multiple functions as companions in perennial cropping systems, but there is a need to screen a wider range of potential species for their suitability.

Many challenges lay ahead on the road to developing a viable perennial crop. Germplasm development itself is not trivial, but the need for novel farming systems adds to the challenge. It is unlikely this challenge will be met by individuals operating in isolation. The *Perennial Wheat Feasibility Study* undertaken in Australia presents as an example of the unexpected success that can be achieved in this challenging field of research particularly where vibrant multi-disciplinary, multi-institutional and global collaboration exists. We contend that future challenges in perennial crop development will be best met by a similar but scaled-up collaborative approach.

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REFERENCES

FAO. 2004. *The history of evolution of triticale. Triticale improvement and production*, by Ammar, K., Mergoum, S. & Rajaram, S. FAO plant production and protection paper 179.

Anderson, D.E. 1961. Taxonomy and distribution of the genus *Phalaris*. *Iowa State Journal of Science*. 36: 1-96.

Angus, J.F. 2001. Nitrogen supply and demand in Australian agriculture. *Australian Journal of Experimental Agriculture*. 41(3): 277-288.

Angus, J.F. & Peoples, M.B. 2012. Nitrogen from Australian dryland pastures. *Crop and Pasture Science*. 63(9): 746-758.

Bell, L.W., Byrne, F., Ewing, M.A. & Wade, L.J. 2008. A preliminary whole-farm economic analysis of perennial wheat in an Australian dryland farming system. *Agricultural Systems*. 96: 166-174.

Bell, L.W. & Moore, A.D. 2012. Integrated crop-livestock systems in Australian agriculture: Trends, drivers and implications. *Agricultural Systems*. 111: 1-12.

Bell, L.W., Moore, A.D. & Kirkegaard, J.A. 2013. Evolution in crop-livestock integration systems that improve farm productivity and environmental performance in Australia. *European Journal of Agronomy*. **In press**.

Berger, L.L. 1992. *Grass tetany: causes and prevention*. Salt Institute report.

Borrill, M. 1972. Studies in *Festuca*. III The contribution of *F. scariosa* to the evolution of polyploids in sections of Bovinae and Scariosae. *New Phytologist*. 71: 523-532.

Boschma, S.P., Lodge, G.M. & Harden, S. 2010. Seedling competition of lucerne in mixtures with temperate and tropical pasture species. *Crop and Pasture Science*. 61(5): 411-419.

Bowman, A.M., Smith, W., Peoples, M.B. & Brockwell, J. 2004. Survey of the productivity, composition and estimated inputs of fixed nitrogen by pastures in central-western New South Wales. *Australian Journal of Experimental Agriculture*. 44(12): 1165-1175.

Brightling, A. 1994. *Stock diseases. Diseases of beef cattle, sheep, goats and farm dogs in temperate Australia*. Chatswood, Australia, Butterworth-Heinemann.

Casburn, G.C., Burns, H.M., Anderson, M., Nugent, T.M., Sproule, P., Olsen, M. & Hayes, R.C. 2013. Crops, rumps and woolly jumpers: An innovative extension approach enabling the complexities of mixed farming to be shared and understood. In: *Proceedings of the 22nd International Grasslands Congress*. 15-19 September 2013, Sydney.

Chen, D., Suter, H., Islam, A., Edis, R., Freney, J.R. & Walker, C.N. 2008. Prospects of improving efficiency of fertiliser nitrogen in Australian agriculture: a review of enhanced efficiency fertilisers. *Soil Research*. 46(4): 289-301.

Cocks, P.S. 2001. Ecology of herbaceous perennial legumes: a review of characteristics that may provide management options for the control of salinity and waterlogging in dryland cropping systems. *Australian Journal of Agricultural Research*. 52(2): 137-151.

Cooper, J.P. 1963. Species and population differences in climatic response. *Environmental control of plant growth*. L. T. Evans. New York, Academic Press: 381-403.

Cottle, D.J. 1991. Digestion and metabolism. *Australian sheep and wool handbook*. D. J. Cottle. Melbourne, Inkata Press: 174-211.

Cox, T.S., Glover, J.D., Van Tassel, D.L., Cox, C.M. & DeHaan, L.R. 2006. Prospects for developing perennial grains. *BioScience*. 56(8): 649-659.

Cox, T.S., Van Tassel, D.L., Cox, C.M. & DeHaan, L.R. 2010. Progress in breeding perennial grains. *Crop and Pasture Science*. 61(7): 513-521.

Culman, S.W., Snapp, S.S., Ollenburger, M., Basso, B. & DeHaan, L.R. 2013. Soil and water quality rapidly responds to perennial grain Kernza Wheatgrass. *Agronomy Journal*. 105: 735-744.

Dann, P., Axelson, A., Dear, B., Williams, E. & Edwards, C. 1983. Herbage, grain and animal production from winter-grazed cereal crops. *Australian Journal of Experimental Agriculture*. 23: 154-161.

Dear, B.S. & Cocks, P.S. 1997. Effect of perennial pasture species on surface soil moisture and early growth and survival of subterranean clover (*Trifolium subterraneum* L.) seedlings. *Australian Journal of Agricultural Research*. 48(5): 683-694.

Dear, B.S., Cocks, P.S., Swan, A.D., Wolfe, E.C. & Ayre, L.M. 2000. Effect of phalaris (*Phalaris aquatica* L.) and lucerne *Medicago sativa* L.) density on seed yield and regeneration of subterranean clover (*Trifolium subterraneum* L.). *Australian Journal of Agricultural Research*. 51(2): 267-278.

Dear, B.S., Moore, G.A. & Hughes, S.J. 2003a. Adaptation and potential contribution of temperate perennial legumes to the southern Australian wheatbelt: a review. *Australian Journal of Experimental Agriculture*. 43(1): 1-18.

Dear, B.S., Peoples, M.B., Cocks, P.S., Swan, A.D. & Smith, A.B. 1999. Nitrogen fixation by subterranean clover (*Trifolium subterraneum* L.) growing in pure culture and in mixtures with varying densities of lucerne (*Medicago sativa* L.) or phalaris (*Phalaris aquatica* L.). *Australian Journal of Agricultural Research*. 50(6): 1047-1058.

Dear, B.S., Sandral, G.A., Peoples, M.B., Wilson, B.C.D., Taylor, J.N. & Rodham, C.A. 2003b. Growth, seed set and nitrogen fixation of 28 annual legume species on 3 Vertosol soils in southern New South Wales. *Australian Journal of Experimental Agriculture*. 43(9): 1101-1115.

Dear, B.S., Sandral, G.A., Virgona, J.M. & Swan, A.D. 2004. Yield and grain protein of wheat following phased perennial grass, lucerne, and annual pastures. *Australian Journal of Agricultural Research*. 55(7): 775-785.

Dear, B.S., Sandral, G.A. & Wilson, B.C.D. 2006. Tolerance of perennial pasture grass seedlings to pre- and post-emergent grass herbicides. *Australian Journal of Experimental Agriculture*. 46(5): 637-644.

Dear, B.S., Virgona, J.M., Sandral, G.A., Swan, A.D. & Orchard, B.A. 2007. Lucerne, phalaris, and wallaby grass in short-term pasture phases in two eastern Australian wheatbelt environments. 1. Importance of initial perennial density on their persistence and recruitment, and on the presence of weeds. *Australian Journal of Agricultural Research*. 58(2): 113-121.

Dear, B.S., Wilson, B.C.D., Rodham, C.A., McCaskie, P. & Sandral, G.A. 2002. Productivity and persistence of *Trifolium hirtum*, *T. michelianum*, *T. glanduliferum* and *Ornithopus sativus* sown as monocultures or in mixtures with *T. subterraneum* in the south-eastern Australian wheat belt. *Australian Journal of Experimental Agriculture*. 42(5): 549-556.

Donald, C.M. 1965. The progress of Australian agriculture and the role of pastures in environmental change. *Australian Journal of Science*. 27(7): 187-198.

Dove, H. 2007. Mineral nutrition of sheep grazing dual-purpose wheats. GRDC-sponsored Grains Research Technical Update. D. Kaminskas and S. Rawlings. Wagga Wagga, NSW, Jon Lamb Communications: ST Peters, SA**:** 71-75.

Dove, H. & McMullen, K.G. 2009. Diet selection, herbage intake and liveweight gain in young sheep grazing dual-purpose wheats and sheep responses to mineral supplements. *Animal Production Science*. 49(10): 749-758.

Erekul, O. & Kohn, W. 2006. Effect of weather and soil conditions on yield components and bread-making quality of winter wheat (*Triticum aestivum* L.) and winter triticale (*Triticosecale* Wittm.) varieties in north-east Germany. *Journal of Agronomy and Crop Science*. 192: 452-464.

FitzGerald, R.D., Wolfe, E.C., Laby, R.H. & Hall, D.G. 1980. Beef production from lucerne and subterranean clover pastures. 2. Bloat occurrence and effect of anti-bloat capsules. *Australian Journal of Experimental Agriculture and Animal Husbandry*. 20(107): 688-694.

Fortune, J.A., Cocks, P.S., Macfarlane, C.K. & Smith, F.P. 1995. Distribution and abundance of annual legume seeds in the wheatbelt of Western Australia. *Australian Journal of Experimental Agriculture.* 35(2): 189-197.

Giunta, F., Motzo, R. & Pruneddu, G. 2003. Comparison of temperate cereals and grain legumes in a Mediterranean environment. *Agricoltura Mediterranea.* 133: 234-248.

Glendinning, J.S. 2000. *Australian Soil Fertility Manual*, revised edition. Collingwood, Vic, CSIRO Publishing.

Glover, J.D., Cox, C.M. & Reganold, J.P. 2007. Future of farming: a return to roots. *Scientific American.* 297(2): 82-89.

Glover, J.D. & Renagold, J.P. 2010. Perennial grains - food security for the future. *Issues in Science and Technology*. Winter (41-47).

Glover, J.D., Reganold, J.P. & Cox, C.M. 2012. Plant perennials to save Africa's soils. *Nature.* 489 (September): 359-361.

Gumbrell. R.C. 1997. Redgut in sheep: A disease with a twist. *New Zealand Veterinary Journal.* 45(6): 217-221.

Hacker, R.B., Hodgkinson, K.C., Melville, G.J., Bean, J. & Clipperton, S.P. 2006. Death model for tussock perennial grasses: thresholds for grazing-induced mortality of mulga Mitchell grass (*Thyridolepis mitchelliana*). *Rangeland Journal.* 28(2): 105-114.

Hackney, B., Dear, B. & Hayes, R. 2006. Summer dormant temperate grasses are productive and persistent in the medium - low rainfall cropping region of New South Wales. *Ground-breaking Stuff - 13th Australian Society of Agronomy Conference.* Perth, The Regional Institute.

Harrington, G.N., Freidel, M.H., Hodgkinson, K. & Noble, K.C. 1984. *Vegetation ecology and management. Management of Australia's rangelands*. G. N. Harrington, A. D. Wilson and M. D. Young. Australia, CSIRO**:** 41-61.

Harrison, M.T., Evans, J.R., Dove, H. & Moore, A.D. 2011. Dual-purpose cereals: can the relative influences of management and environment on crop recovery and grain yield be dissected? *Crop and Pasture Science*. 62(11): 930-946.

Hayes, R.C., Dear, B.S., Li, G.D., Virgona, J.M., Conyers, M.K., Hackney, B.F. & Tidd, J. 2010a. Perennial pastures for recharge control in temperate drought-prone environments. Part 1: productivity, persistence and herbage quality of key species. *New Zealand Journal of AgriculturalResearch.* 53(4): 283-302.

Hayes, R.C., Dear, B.S., Orchard, B.A., Peoples, M.B. & Eberbach, P.L. 2008. Response of subterranean clover, balansa clover, and gland clover to lime when grown in mixtures on an acid soil. *Australian Journal of Agricultural Research*. 59(9): 824-835.

Hayes, R.C., Li, G.D., Dear, B.S., Conyers, M.K., Virgona, J.M. & Tidd, J. 2010b. Perennial pastures for recharge control in temperate drought-prone environments. Part 2: soil drying capacity of key species. *New Zealand Journal of Agricultural Research*. 53(4): 327-345.

Hayes, R.C., Newell, M.T., DeHaan, L.R., Murphy, K.M., Crane, S., Norton, M.R., Wade, L.J., Newberry, M., Fahim, M., Jones, S.S., Cox, T.S. & Larkin, P.J. 2012. Perennial cereal crops: An initial evaluation of wheat derivatives. *Field Crops Research*. 133: 68-89.

Herridge, D. 2011. *Managing legume and fertiliser N in northern grains cropping*, Grains Research and Development Corporation.

Hubble, G.D., Isbell, R.F. & Northcote, K.H. 1983. *Features of Australian soils. Soils: an Australian viewpoint*. Melbourne, Division of Soils, CSIRO**:** 17-47.

Hutchings, T.R. & Nordblom, T.L. 2011. A financial analysis of the effect of the mix of crop and sheep enterprises on the risk profile of dryland farms in south-eastern Australia. *Australian Farm Business Management Journal.* 8(1): 19-42.

Jaikumar, N.S., Snapp, S.S., Murphy, K. & Jones, S.S. 2012. Agronomic assessment of perennial wheat and perennial rye as cereal crops. *Agronomy Journal*. 104(6): 1716-1726.

King, W.M., Dowling, P.M., Michalk, D.L., Kemp, D.R., Millar, G.D., Packer, I.J., Priest, S.M. & Tarleton, J.A. 2006. Sustainable grazing systems for the Central Tablelands of New South Wales. 1. Agronomic implications of vegetation–environment associations within a naturalised temperate perennial grassland. *Australian Journal of Experimental Agriculture*. 46(4): 439-456.

Kirkegaard, J.A., Sprague, S.J., Hamblin, P.J., Graham, J.M. & Lilley, J.M. 2012. Refining crop and livestock management for dual-purpose spring canola (*Brassica napus*). *Crop and Pasture Science*. 63(5): 429-443.

Lane, L.A., Ayres, J.F. & Lovett, J.V. 2000. The pastoral significance, adaptive characteristics, and grazing value of white clover (*Trifolium repens* L.) in dryland environments in Australia: a review. *Australian Journal of Experimental Agriculture*. 40(7): 1033-1046.

Larkin, P.J. & Newell, M.T. 2014. Perennial wheat breeding: Current germplasm and a way forward for breeding and global cooperation. In: C. Batello, L. Wade, S. Cox, N. Pogna, A. Bozzini, J. Choptiany (eds), *Proceedings of the FAO Expert Workshop on Perennial Crops for Food Security*, 28-30 August 2013, Rome, Italy, p 39-53.

Li, G.D., Lodge, G.M., Moore, G.A., Craig, A.D., Dear, B.S., Boschma, S.P., Albertson, T.O., Miller, S.M., Harden, S., Hayes, R.C., Hughes, S.J., Snowball, R., Smith, A.B. & Cullis, B.R. 2008. Evaluation of perennial pasture legumes and herbs to identify species with high herbage production and persistence in mixed farming zones in southern Australia. *Australian Journal of Experimental Agriculture*. 48: 449-466.

Llewellyn, R., Robertson, M., Hayes, R., Ferris, D., Revell, C. & Descheemaeker, K. 2013. A strategic approach to developing the role of perennial forages for crop-livestock farmers. In *Proceedings of the 22nd International Grasslands Congress*. 15-19 September 2013, Sydney.

Love, G. 2004. *Impacts of climate variability on regional Australia*, Australian Beureau of Agricultural and Resource Economics.

Lumaret, R. 1988. Cytology, genetics and evolution in the Genus *Dactylis*. *Critical Reviews in Plant Sciences*. **7**: 55-91.

Malinowski, D.P., Zuo, H., Kramp, B.A., Muir, J.P. & Pinchak, W.E. 2005. Obligatory summer-dormant cool-season perennial grasses for semiarid environments of the Southern Great Plains. *Agronomy Journal*. 97: 147-154.

Martinek, P., Vinterova, M., Buresova, I. & Vyhnanek, T. 2008. Agronomic and quality characteristics of triticale (X *Triticosecale* Wittmack) with HMW glutenin subunits 5+10. *Journal of Cereal Science*. 47(1): 68-78.

McMullen, K.G. & Virgona, J.M. 2009. Dry matter production and grain yield from grazed wheat in southern New South Wales. *Animal production Science*. 49: 769-776.

Mergoum, M., Pfeiffer, W.H., Pena, R.J., Ammar, K. & Rajaram, S. 2004. *Triticale crop improvement: the CIMMYT programme*. *Triticale improvement and production*. FAO plant production and protection paper 179. M. Mergoum and H. Gomez-Macpherson. Rome, Italy, Food and Agriculture Organization of the United Nations.

Mills, A., Moot, D.J. & McKenzie, B.A. 2006. Cocksfoot pasture production in relation to environmental variables. In *Proceedings of the New Zealand Grassland Association*. 68: 89-94.

Moore, A.D. 2009. Opportunities and trade-offs in dual-purpose cereals across the southern Australian mixed-farming zone: a modelling study. *Animal Production Science*. 49(10): 759-768.

Motzo, R., Bassu, S. & Giunta, F. 2011. Variation for kernel number and related traits in triticale (x *Triticosecale* Wittmack). *Crop and Pasture Science*. 62: 823-829.

Neal-Smith, C.A. 1955. *Report on herbage plant exploration in the Mediterranean region*. Rome, F.A.O.

Newell, M., Hayes, R. & Larkin, P. 2013. Perennial cereals: A novel source of feed for grazing livestock. *22nd International Grasslands Congress*. Sydney, Australia, In press.

Nichols, P.G.H., Loi, A., Nutt, B.J., Evans, P.M., Craig, A.D., Pengelly, B.C., Dear, B.S., Lloyd, D.L., Revell, C.K., Nair, R.M., Ewing, M.A., Howieson, J.G., Auricht, G.A., Howie, J.H., Sandral, G.A., Carr, S.J., de Koning, C.T., Hackney, B.F., Crocker, G.J., Snowball, R., Hughes, S.J., Hall, E.J., Foster, K.J., Skinner, P.W., Barbetti, M.J. & You, M.P. 2007. New annual and short-lived perennial pasture legumes for Australian agriculture - 15 years of revolution. *Field Crops Research*. 104(1-3): 10-23.

Nichols, P.G.H., Revell, C.K., Humphries, A.W., Howie, J.H., Hall, E.J., Sandral, G.A., Ghamkhar, K. & Harris, C.A. 2012. Temperate pasture legumes in Australia—their history, current use, and future prospects. *Crop and Pasture Science*. 63(9): 691-725.

Norton, M.R. & Koetz, E.A. 2013. Cover cropping and establishment of lucerne-based pastures in a semi-arid zone, mixed farming system of uniform rainfall distribution. *Grass and Forage Science*. 68: 1-14.

Norton, M.R., Lelièvre, F. & Volaire, F. 2006a. Summer dormancy in *Dactylis glomerata* L.: the influence of season of sowing and a simulated mid-summer storm on two contrasting cultivars. *Australian Journal of Agricultural Research*. 57(5): 565-575.

Norton, M.R., Lelièvre, F. & Volaire, F. 2012. Summer dormancy in *Phalaris aquatica* L., the influence of season of sowing and summer moisture regime on two contrasting cultivars. *Journal of Agronomy and Crop Science*. 198(1): 1-13.

Norton, M.R., Volaire, F. & Lelièvre, F. 2006b. Summer dormancy in *Festuca arundinacea* Schreb.; the influence of season of sowing and a simulated mid-summer storm on two contrasting cultivars. *Australian Journal of Agricultural Research*. 57(12): 1267-1277.

Oram, R.N. 1990. *Register of Australian herbage plant cultivars*, third edition. Canberra, CSIRO Division of Plant Industries.

Oram, R.N., Ferreira, V., Culvenor, R.A., Hopkins, A.A. & Stewart, A. 2009. The first century of *Phalaris aquatica* L. cultivation and genetic improvement: a review. *Crop and Pasture Science*. 60(1): 1-15.

Peoples, M.B., Brockwell, J., Hunt, J.R., Swan, A.D., Watson, L., Hayes, R.C., Li, G.D., Hackney, B., Nuttall, J.G., Davies, S.L. & Fillery, I.R.P. 2012. Factors affecting the potential contributions of N_2 fixation by legumes in Australian pasture systems. *Crop and Pasture Science*. 63(9): 759-786.

Picasso, V.D., Brummer, E.C., Liebman, M., Dixon, P.M. & Wilsey, B.J. 2008. Crop species diversity affects productivity and weed suppression in perennial polycultures under two management strategies. *Crop Science*. 48(1): 331-342.

Picasso, V.D., Brummer, E.C., Liebman, M., Dixon, P.M. & Wilsey, B.J. 2011. Diverse perennial crop mixtures sustain higher productivity over time based on ecological complementarity. *Renewable Agriculture and Food Systems*. 26(4): 317-327.

Ridley, A.M. & Simpson, R.J. 1994. Seasonal development of roots under perennial and annual grass pastures. *Australian Journal of Agricultural Research*. 45(5): 1077-1087.

Russelle, M.P., Entz, M.H. & Franzluebbers, A.J. 2007. Reconsidering integrated crop-livestock systems in North America. *Agronomy Journal*. 99(325-334).

Salehi, M. & Arzani, A. 2013. Grain quality traits in triticale influenced by field salinity stress. *Australian Journal of Crop Science*. 7(5): 580-587.

Salmanowicz, B.P. & Dylewicz, M. 2007. Identification and characterisation of high-molecular-weight glutenin genes in Polish titicale cultivars by PCR-based DNA markers. *Journal of Applied Genetics*. 48(4): 347-357.

Scott, J.F., Lodge, G.M. & McCormick, L.H. 2000. Economics of increasing the persistence of sown pastures: costs, stocking rate and cash flow. *Australian Journal of Experimental Agriculture*. 40(2): 313-323.

Stork, P.R. & Jerie, P.H. 2003. Initial studies of the growth, nitrogen sequestering, and de-watering potential of perennial grass selections for use as nitrogen catch crops in orchards. *Australian Journal of Agricultural Research*. 54(1): 27-37.

Tian, L.H., Bell, L.W., Shen, Y.Y. & Whish, J.P.M. 2012. Dual-purpose use of winter wheat in western China: cutting time and nitrogen application effects on phenology, forage production, and grain yield. *Crop and Pasture Science*. 63(6): 520-528.

Tracey, B.F. & Sanderson, M.A. 2004. Productivity and stability relationships in mowed pasture communities of varying species composition. *Crop Science*. 44: 2180-2186.

USDA. 2013. *World Agricultural Production*. (Available at www.fas.usda.gov/wap/current/default.asp).

Virgona, J.M. & Dear, B.S. 1996. Comparative performance of Caucasion clover (*Trifolium ambiguum* cv. Monaro) after 11 years under low-input conditions in south-eastern Australia. *New Zealand Journal of Agricultural Research*. 39: 245-253.

Virgona, J.M., Gummer, F.A.J. & Angus, J.F. 2006. Effects of grazing on wheat growth, yield, development, water use, and nitrogen use. *Australian Journal of Agricultural Research*. 57(12): 1307-1319.

Volaire, F. 1995. Growth, carbohydrate reserves and drought survival strategies of contrasting *Dactylis glomerata* populations in a Mediterranean environment. *Journal of Applied Ecology*. 32: 56-66.

Volaire, F. & Conejero, G. 2001. Drought survival and dehydration tolerance in *Dactylis glomerata* and *Poa bulbosa*. *Australian Journal of Plant Physiology*. 28: 743-754.

Volaire, F. & Norton, M. 2006. Summer dormancy in temperate grasses. *Annals of Botany*. 98: 927-933.

White, J. & Edwards, J. 2008. *Wheat growth and development*. Orange, NSW Department of Primary Industries.

Wilkins, R.J. 2008. Eco-efficient approaches to land management: a case for increased integration of crop and animal production systems. *Philosophical Transactions of the Royal Society of British*. 363 (517-525).

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BACK TO THE FUTURE! THOUGHTS ON RATOON RICE IN SOUTHEAST AND EAST ASIA

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In China *Oryza sativa* has been grown as an annual for perhaps 10-12 000 generations although ratooning certainly survived until about 3 500 years ago. By contrast, in Southeast Asia, including marginal areas populated mainly by ethnic minority peoples such as India's northeastern territories and parts of Bangladesh, the cultivation of perennial strains extended into the twentieth century. The degree to which this practice survives to the present is not known. Despite being grown as an annual, many strains of rice retain a perennial habit to some degree though yields are commonly very much lower from ratoon crops than from initial plantings. In Japan, a ratoon yield of about 15 percent of the first harvest has been reported. No systematically-gathered data on ratoon-crop yields have been found, though Hill (2010) has drawn together historical accounts of the practice. He reported observing it in Johor, Peninsular Malaysia in the 1960s and in northern Laos in the 2010s.

The need for Asian rice-growers to move from highly labour-intensive methods to less labour-intensive methods arises from a general rise in the cost of labour. In the 1960s the opportunity cost of rice-growing in China and much of Southeast Asia was probably close to zero. In most of the region it is now much above that level, a situation reflected in substantial short-term circular migration by rice-growers and in some cases by production at an economic loss, as was already reported in parts of Peninsular Malaysia in the 1960s.

Because tillage, nursery-preparation and planting, and, especially, transplanting, may require half to two-thirds of labour input per crop, any system of production that can reduce such inputs, without an excessive yield penalty is very desirable for the cost of labour will inevitably continue to rise.

Keywords: ratooning rice, Southeast Asia, East Asia, agricultural development

INTRODUCTION

In Asia rice ratooning has a long history, one which is generally little known among rice scientists or farmers. For Southeast Asia, Hill (2010) has examined that history in some detail, pointing out that much of the documentary record has been misinterpreted by later commentators. This paper extends the analysis to China and Japan though for linguistic reasons this author does not have access to works in Japanese or in Chinese. Drawing on the resources in his on-line bibliography on the history of Southeast Asian agriculture (Hill, 2007), an outline of the historical record for the region is given. This is followed by a consideration of some important areas for the future study of ratooning and assessment of the feasibility of promoting ratooning in the region.

Over the last half century the region has seen a remarkable structural transformation of agriculture in general and rice production in particular. Generally there has been a long-continued process of commercialization of production, though in some areas this has had limited effects, largely because of structural limitations in production, such as very small size of farms and, especially limited alternative activities. Fifty years ago it seems likely that in much of the region, Japan and Southeast Asian plantation areas accepted the opportunity cost of rural farm labour was close to zero. That situation has largely changed with urban employment as a rapidly-emerging economic alternative. This has been and continues to be linked with permanent rural-urban migration but also with widespread temporary circular migration. For example, a study some years ago showed that the population of Bangkok in the dry season was about nine percent higher than in the wet season. This was the result of farmers flocking to the towns for temporary employment, partly in manufacturing but especially in construction, as the Thai case suggests (Hill, 2002).

Urbanization and the overall growth in real incomes together with demographic changes have also had the effect of reducing per person demand for rice, though total demand has continued to rise partly for demographic reasons. This situation is unlikely to last. The population fertility rates of Japan and Thailand, as well of major urban concentrations such as Hong Kong and Singapore, are now well below replacement level which is about 2.2 children per woman of child-bearing age. China's population growth rate is forecast to fall to zero around 2026 and the total population will fall substantially thereafter unless its government abandons its 'one-child' policy and adopts a more pro-natalist stance. Even if it does that there is likely to be a substantial increase in the cost of labour for around two decades until the new generation reaches the labour force.

Globally, the consumption of rice per person has levelled out the late 1980s (Rejesus *et al.* 2012) though demand in Africa continues to rise. Estimates of very large increases in demand are probably not well-founded. Fageria (2007), for example, estimated a requirement of 60 percent more rice by 2025, just over a decade away. The reality is that since the 2007-8 season, global rice stocks have tended to rise, reaching close to an estimated 35 percent of annual global consumption by 2013-14 (FAO Rice Monitor, July 2013). This will give something of a breathing space to develop alternatives to the region's current highly labour-intensive methods.

At the same time, an emerging consideration in the production of rice is urban expansion, in many areas onto prime rice-growing land. Politically, governments continue to be faced with a need to ensure a continued supply of rice to urban markets at reasonable prices. Every government in the region is aware of the need to hold rice prices at a reasonable level for urban workers. Given that farm labour costs are inevitably rising and that labour mobility is increasing, there is a need to control the costs of rice production. One method of doing this is to ratoon, for this approach substantially reduces the labour cost of traditional methods involving nursery preparation and transplanting, probably by around 50 to 60 percent per crop (Flinn and Mercado, 1988). One competing strategy, of course, is to abandon transplanting and to substitute for it broadcast sowing. However, this has the considerable disadvantage that satisfactory weed control in the early stages of growth requires enhanced applications of herbicides, the long-term effects of which are not fully-known. This may emerge as an issue with ratooning as well, especially if a main crop is followed by two ratoons, as seems to have been practice in some areas in the past.

RATOONING - THE HISTORICAL RECORD

Ratooning clearly has a long history. In China, so far considered to be the home of the longest-running sequence of rice cultivation, it seems likely that ratooning was abandoned as a general practice in early historical times, perhaps 3 000 years ago or even more. If this is so, then the practice of growing *Oryza sativa* as an annual may have led to genetic drift away from good yields from ratoons. Certainly, the limited data for ratoon yields from present-day varieties show a wide range. An analysis of such literature as is available to me gives claimed ratoon yields ranging from around 8.7 tonnes/ha (Xu *et al.* 1988; Prashar, 1970) to about 0.3 tonnes/ha or even less. Chauhan *et al.* (1988) give comprehensive data. Parenthetically, it should be noted here that almost without exception writers on the subject of yields fail to give data on the size of the plots employed in making their yield estimates. Many are probably serious over-estimates, seemingly being based upon small-scale trials.

The origins of rice cultivation have been the subject of much debate, some of it perhaps underlain by nationalistic considerations. Oka and Morishima (1997) review several hypothesized routes to the evolution of Oryza *sativa*, pointing out that many common wild rice varieties tend to differentiate into *indica* and *japonica* types. Watanabe (1997) briefly examines the origin and differentiation of cultivated rice in Asia. As a crop, rice may go back 6-8 000 years in China though whether it was fully-domesticated at that time is a matter of some doubt (Sweeney and McCouch, 2007; Liu Zhiyi, 2000). Similar ages have been claimed for India. Rice-growing in Japan dates back to the late Jomon period, around 3 000 BP at the earliest (Matsuo *et al.* 1997). This is somewhat later than the earliest rice in mainland Southeast Asia where the crop dates back four or five millennia, possibly more. Even in equatorial Southeast Asia, the crop may date back as much as six millennia, as recent data from the Niah Cave, Sarawak, suggest (Hunt and Rushworth, 2005). Their finding at this low latitude, just south of four degrees north latitude, may imply an early existence of non-photoperiodic varieties or at least of varieties responsive to very small differences in day-length. What can be asserted with some degree of confidence is that *O. sativa* probably differentiated into two subspecies, the more northerly and temperate *japonica* and the more equatorial *indica*, as a result of at least two independent series of steps leading to domestication (Tao Sang and Song Ge, 2007).

Arguably, many of the early varieties of rice in the region had a significant ability to ratoon though wherever it may have been grown it seems likely that it would not have been grown beyond a second ratoon at the most, for by that stage the competition from weeds would probably have rendered yields so low as to be not worth harvesting. A search of the modern literature failed to find a single case of anything beyond a first ratoon, though as I have argued elsewhere, it seems likely that a second ratoon was probably taken in Indochina and in other parts of Southeast Asia in earlier historical times (Hill, 2010). Documentary and field research has shown that the practice of ratooning survived into modern times in the Malay Peninsula, in Laos, and reportedly, in one-crop areas in Japan (T.S. Stanley, personal communication, 10 Dec. 2007).

Earlier, ratooning seems to have been fairly widespread. While not quite a ‘free good’, ratoon rice avoids the need to till the soil, to prepare nurseries and to transplant seedlings to the extent that this practice may reduce labour demand by about half. Certainly it may increase the labour demand for weeding but not to a level comparable to the demands of soil preparation, nursery preparation and transplanting. For China, Ho Ping-ti has assembled firm evidence for what was probably perennial cultivation, likely more or less contemporaneous with annual cultivation, dating from the Shang dynasty (*ca* 1 600 BC to *ca* 1 046 BC), though Ho refers to it as a wild rice (Ho, 1957, 1969). Cultivation of some kind, or at least weeding and replanting are probably indicated because weed invasion inevitably overtakes any abandoned rice-field. Fuller, Harvey and Qin (2007) have pointed to the cultivation of what they rather paradoxically refer to as 'wild' rice, as early as the 5 000 BC.

The documentary record for Southeast Asia is rather more extensive though bedevilled by major gaps, for example for Indonesia. Clercq (1871) is just one of a host of papers in Dutch on agricultural practices in colonial times in Indonesia to be silent on the matter of rice ratooning. It is unlikely to have been altogether absent. For Japan the evidence for ratooning at any period linguistically accessible to this author is exiguous. The four-volume compilation by Matsuo and his colleagues seemingly makes no mention of the practice though it is difficult to be certain because that work lacks an index. Papers in that collection make no mention of the practice (Matsuo *et al.* 1997).

The early literature has been beset by problems of interpretation, as Hill, (2010) has noted. In particular, in archaeological contexts, is the formidable difficulty of distinguishing the remains of annually-grown rice varieties from their perennial cousins. What is clear is that much of the work of historians of the region dealing with the documentary evidence has been bedevilled by a lack of knowledge of field practice by present-day cultivators. It is simply beyond belief that the rice-growers of thirteenth-century Cambodia had the means to complete three or four full cropping cycles in a year for even today, two are not common, depending as they do upon an adequate supply of irrigation water. The notion of three 'crops' in a year is also to be found in Chapman's account of Cochin China in the late eighteenth century but again the probability must be that this refers to three harvests rather than to three full crop cycles (see Lamb, 1961). If this account be a little equivocal, that of Father Pierre Poivre for Siam, published in 1770, very likely refers to ratooning though an alternative explanation is that the rice was a shattering variety.

> *'It is astonishing, however, to observe, these lands, frequently neither laboured nor sown for years together, produce extraordinary crops of rice. The grain, reaped negligently, sows of itself, and reproduces [sic.] annually another harvest, by the help of the river Menam....' (Poivre, 1770).*

Another early account is that of Ma Huan for Java in the early fifteenth century. He noted that rice ripened twice in a year and that the kernels were small. The latter observation is probably a clincher for it is now known that the grains of perennial varieties tend to be smaller, on average, than those of more annual varieties. Other examples are quoted by Hill (2012). In seventeenth century Siam, now Thailand, Nicholas Gervaise reported in 1688, 'One sort that grows without anyone sowing it...' Perennial though it must have been, however, it could not have survived colonization by adventitious vegetation but for human intervention. A century or so later the Abbé Raynal spoke of rice that 'bore plentiful crops spontaneously' – surely again a reference to a perennial variety. More equivocal is an account of Assam by Neufville dating from the early nineteenth century. He spoke of the lowlands producing two crops annually, possibly referring to a main crop and a ratoon (Neufville, 1828).

Rather later is a report for the Philippines by Alfred Marche who travelled in that region in 1879 to 1881. Like the others already mentioned, he reported up to three harvests in a year in Laguna Province, with parts of Tarlac and Pampanga, the location of dry-season harvesting described 40 years later by Apostol.

Even more recent are several accounts of a small area in what is now Arunachal Pradesh by the German, later British, ethnographer Christoph von Fürer-Haimendorf (1946, 1955, 1962). He described two types of rice-fields at an elevation of about 1 500 metres – those kept permanently wet and those that allowed to dry out soon after harvest. On the former class of land the soil was not tilled, the rice being perennial though where there were gaps in the plant cover these were made good by the planting of seedlings early in the growing season. Von Fürer-Haimendorf's 1962 paper speaks as if this form of cultivation still existed but whether it still survives and whether there are holdings of the ratooned rice varieties in any repository are not known.

This author has seen ratooning in the field for consumption as food only once. In the early 1960s he visited the Orang Kanaq, a small group of aboriginal people whose ancestors were settled in Johor from the Indonesian province of Riau. They no longer grow the crop (Mahani Musa, 2011). On a much later visit to a rural area east of the northern Lao town of Vientiane some ten years ago, ratooning was again seen but then it was unlikely that the crop was being harvested, for the area was being grazed by cattle, a practice widespread in most of SE Asia before double-cropping became common.

RATOONING – THE PRESENT SITUATION

The modern literature on the ratooning of rice is quite scattered. A good deal relates to India rather than to East and Southeast Asia though much of that is relevant because it deals with general agronomic matters of wide applicability. A useful starting point is the IRRI collection of essays *Rice ratooning* (IRRI, 1988), though the appearance of that monograph, the reportage has increased steadily. Basically, a ratoon crop has the major advantages over a transplanted crop of requiring only about half of the labour input of the main crop and perhaps 60 percent less water (Oad *et al.* 2002; Oad *et al.* 2002). There is, however, a very wide range of genetic potential for ratooning with some cultivars giving very small yields, or none, and others giving yields that are greater than the main-crop yield of the same cultivar. (see, for example, Krishnamurthy, 1988).

Incidentally it may be noted in this context that seasonality may play a part here. Many research reports fail to mention the obvious point that in theory a proper comparison of main-crop (transplanted) and ratoon yields requires that the crops be compared over the same time-period, a condition not readily met given the much shorter growing period of the ratoon. Replication over several seasons may reduce errors of estimation arising from this source.

While there is some lack of knowledge among present-day rice scientists that ratooning has been of some significance in the more-distant past, there is a small body of publications on the subject, mainly by Indian workers, dating from the 1970s. (see *Rice ratooning*, 1988, for examples, especially papers by Krishnamurthy and by Mahadevappa, for overviews). There is a small literature by Chinese workers, mainly in Chinese. For Japan there seems to be very little literature, at least in English or any other western language. Ichii and Kuwada's paper of 1981 and some of their references are exceptions. The major four-volume work edited by Matsuo *et al.* (1997), a translation from Japanese into English, seemingly makes no mention of the practice though it is difficult to be certain for the work is not indexed. The standard international work on the subject, the IRRI *Rice ratooning*, 1985, is now rather dated but brings together a good deal of what rice scientists were investigating at that point.

Although there is a considerable body of modern literature on ratooning, some of its value is reduced by deficiencies in research methodology and reportage. An early paper by Prashar (1970) for example, compared the ratoon and main crop yields of two modern HYV's, IR 5 and IR 8, reporting remarkably high yields ranging from 6 tonnes per hectare to almost nine, with IR 8 outperforming the earlier cultivar. As with many later studies, it may be suspected that the yield data are derived from very small scale cutting trials.

The study by Ichii and Kuwada (1981) gave yields for ratoons harvested at varying intervals with the highest yields at 10 and 20 days after heading but fail to give the areal unit to which they refer. Many papers also fail to give details of the plot size to which their data refer. This is a considerable weakness for it has long been known that reported yields from square-metre scale experiments often far outweigh those from plantings at larger scales. Xu *et al.* (1988) for instance state that their results 'were obtained from small areas' but fail to indicate how small. Their results therefore suffer from the common defect of such studies as giving unrealistically high yields. They give main crop yields ranging from 5.6 to 9.8 tonnes/ha and ratoon yields from 3.1 to 8.7 tonnes/ha, in one case, for IR 24, with a ratoon yield of 8.7 tonnes/ha/day with a main crop of 8.4 tonnes/ha.

More comprehensive data, covering 124 experimental plantings, many in India, are those of Chauhan *et al.* (1988). Outstanding were ratoon performances by the variety Intan, reported from Karnataka, India, at 2.3 to 7.7 tonnes/ha, the variety Milbuen 5 from the Philippines, at 5.6 tonnes/ha, and IR 8 at 8.2 and 8.7 tonnes/ha, all above the main crop yields. By contrast, moderate ratoon yields were reportedly obtained from IR 42 and IR 97523-71-3-2, ranging from 33 to 49 percent of the main crop yields with ten cultivars giving a ratoon yield of less than 10 percent of the main crop yields. One early comparison of IR 5 and IR 8 is that of Prashar (1970) for Ethiopia. He claimed that IR 8 outyielded IR 5 for both the main and ratoon crops though his yield data, ranging from 6.3 to 8.7 tonnes/ha, like many others, may be suspect.

Another relevant paper is that of Chauhan *et al.* (1988). These workers screened 24 modern genotypes and found that of the 24 examined, only ten showed any regeneration at all, with RP 1664-4461 showing a very modest ratoon yield of 1.7 tonnes/ha and IET 7613 a yield of only 0.8 tonnes/ha. This result raises the suspicion that ratooning ability may have been bred out of some of the modern cultivars. If this notion is sustained, important considerations are raised as a strategy for future research is developed. Of particular concern is the fact that IRRI has screened for their ratooning ability only a tiny proportion of its vast holdings of cultivars.

On the other hand, work in Karnataka, India, with six modern cultivars, including IR 28, showed excellent yields from both the main crop and the ratoon (Krishnamurthy, 1988). Main crop yields reportedly ranged between 8.7 and 11.8 tonnes/ha for the main crop. In percentage terms the ratoon yield ranged between 67 and 90 percent of the main-crop outturn where the main crop had been direct-seeded, compared with a range of ratoon yields between 59 and 78 percent of main crop yields where the main crop had been transplanted. A later study of lowland genotypes, by Santos *et al.* (2003), involved five early maturing modern varieties and four medium-term types. For the former the average ratoon yield was 59 percent of the main crop outturn but for the latter types the average was a disappointing 39 percent.

Flinn and Mercado (1988) have a most useful overview of the economic aspects of ratooning, concluding that the technique offers major advantages by reducing both labour and water requirements by about half compared with the main transplanted crop. Another advantage is the reduced length of the crop year, opening the possibility of a further crop, other than rice in the same crop year, and the freeing up of labour and other resources for alternative uses. This is a particular advantage where temporary circular migration and the earnings from urban employment have become important. But these authors also point to economic disadvantages. Included are uneven maturing of the ratoon crop, uneven grain quality and generally low and uncertain yields, matters of no great concern where production is for subsistence perhaps, but important where the crop is marketed.

The question of whether or not technical innovations are gender-neutral is one of considerable importance. It is widely-known in Peninsular Malaysia and Indonesia, for example, that beginning in the 1960s, the harvesting of rice panicle by panicle over the course of several weeks by women using the traditional small harvesting knife was replaced by men wielding sickles. Given that in the major rice-growing states of northern Peninsular Malaysia and in nearby Peninsular Thailand, gangs of women were employed as harvesters, this was a severe loss of income in some villages of that region. One further consequence was that quality immediately fell as immature panicles were cut together with the mature ones. In turn that necessitated much closer attention to field levels since uneven ripening in part reflected variations in soil moisture across the fields (Baker, 1940; Colani, 1940; Fukuda, 1986). In the Minangkabau areas of Peninsular Malaysia, where little rice-growing still survives, the introduction of machine tillage in the 1960s had a reverse effect. There tillage by women, who mostly owned the land, was gradually replaced by men driving hand tractors.

A further clear advantage of ratooning may be added. For regions frequently vulnerable to damage from tropical cyclones, notably the Philippines north of Mindanao, the southern provinces of China within about 100 km of the sea, and the central and northern provinces of Viet Nam, ratooning potentially reduces the length of the growing season compared to double-cropping thus avoiding the effects of late-season cyclones. In this context it is worth noting that studies of climate change are forecasting an increase in the number and intensity of tropical cyclones, probably also to be accompanied by more, and more intense rain.

Since 1988, understanding of some of the 'mechanics' of ratooning has increased. For example, a Texas study by Turner and Jund (1993) showed that good levels of total non-structural carbohydrate (TNC) in the main crop were essential to satisfactory yield from the ratoon. They also suggest that cultivars may differ widely in their ability to accumulate TNC prior to heading. Both findings have been confirmed for an Asian context by Cheng and Li (1994) who also noted that only one of the five *indica* hybrids they examined showed good ratooning ability.

One area of research that has attracted some attention is that of the optimal height for cutting the culms of the main crop to ensure a good yield from the ratoon. This is because the ratoon yield depends upon the total carbohydrate content in the stem base (Oad *et al.* 2002a,b). A Texas study by Jones (1993) suggested that ratoon yields for the two American varieties used, 'Lebonnet' and 'Lemont', could be optimized by lowering the cutting height of the main crop to 20 - 30 cm. Other authors, with South American or Asian experience, suggest that the optimal level may be somewhat lower at 10 - 20 cm (see Santos *et al.* 2003, and for example, Bahar and De Datta, 1977; Calendacion *et al.* 1992). Ahmed and Das's work (1988) rather contradicts that finding for they noted that ratoon yields remained about the same for heights from 15 - 45 cm but declined drastically below the lower level. An earlier study, by Prashar (1970), showed quite a contrary pattern. He found that the ratoon yield was significantly higher where the main crop was cut at ground level rather than at four, eight and 12 cm, though the maturity period was shorter with higher cutting. Clearly, as with many other characteristics, there is considerable variability but it seems likely that cutting the main crop stems at a low level, can, other things being equal, be compensated by a delay in harvesting. That, of course, raises issues of reliable water supply and in climatically marginal areas, sufficient warmth to continue growth.

One issue that has received rather limited consideration is that of the quality of the ratoon crop, not a major consideration where the crop is for self-consumption by the cultivator and his family but an important issue for the commercial and semi-commercial producer because lower quality means lower income. No reportage on the physiology of ratooning that may lead to uneven ripening has been found.

Part of the problem is asynchronous ripening of the ratoon (Calendacion *et al.* 1992). This is certainly so where, as is general in commercial production, harvesting is done in a few hours rather than over weeks. That was once general practice in many parts of insular Southeast Asia. At lower latitudes in Southeast Asia, panicle-by-panicle harvesting using a small knife was general until the 1960s though it has now been largely replaced by the sickle and a single harvest. Practised only in single-crop areas, that method meant that harvesting could be spread over as much as two months so that variable ripeness was much less an issue. Presumably, were that method to be applied to the ratoon crop, the problem of uneven ripening might be mitigated, but only at the cost of a considerable increase of labour input, one so large as to make that approach unattractive to commercial producers.

CONCLUSION

Just how widespread ratooning may currently be is difficult to establish. For the Philippines, for example, it has been claimed that more and more farmers gain extra income from ratooning, especially in Bulacan and Nueva Ecija provinces (Lacanlale, 2004). One newspaper report indicates that in Leyte 5 000 ha of potential ratoon-crop land has been identified (*Sun Star* 17 July 2013). But for most of the region, good data are lacking. In Malaysia, for example, where rice-growing is heavily subsidized by government, the problem of the rising cost of agricultural labour has been met from two sources. One is the growing practice of broadcast sowing, requiring the enhanced application of selective herbicides, and the other is by the importation of low-paid field labour from outside the country. In this context, a study of the costs and benefits of this approach compared with ratooning is desirable. This might include consideration of the social costs of such migrant labour.

For farmers a key question is whether to ratoon or not. On this issue the size of the main-crop harvest is not a good indicator, for the key question is the level of TNC – total non-structural carbohydrates – in the stems of the main crop. A high level means that, other things being equal, it is safe to proceed with ratooning (Boyd, 2000). This test offers reinforcement to the rather subjective method of observing the speed at which stubble was regrowing after the main-crop harvest. By lowering the main-crop cutting height to about 20 cm d with the usual 45 cm, it has been found that the ratoon yield is enhanced quite substantially, to the extent of 1.1 to 3.3 tonnes/ha as reported by Boyd for Texas. So far as is known, no such test is available in Asia.

One novel approach is that of Calendacion (1992) and his colleagues. They deliberately flattened the standing straw after the main crop harvest thereby locking it prone upon the soil surface, an action they term 'lock-lodging'. This was done manually. At a mean of about 1.5 tonnes/ha, yields from plots thus treated were significantly higher than from conventional ratooning at about 1.1 tonnes/ha, though otherwise the treatments were the same. This procedure requires more labour than conventional ratooning. Perhaps a similar effect might be achieved by the application of a heavy roller, perhaps a toothed type, to improve aeration on heavy clay soils especially.

Clearly, one thing that must be avoided at all costs is the kind of rice development debacle represented by attempt to develop a million hectares of rice land from forest in Kalimantan

(Boehm and Siegert, 2001; Rieley, 2001). This project, launched in 1995, aimed at the development of what is mainly peat land, from the outset, a very problematic undertaking. It ultimately directly affected some 1.5 million hectares, while burning in 1997 is estimated to have covered 15 million km^2 in smoke for a period of several weeks and to have added 0.5 parts per million CO_2 to the global atmosphere (Rieley, 2001).

Ratooning must be a viable alternative to that approach. The Philippines government is promoting it as a means of attaining national self-sufficiency in rice (*Sun Star* newspaper, 17 July 2013), though to this observer, the estimate of only 45 days to obtain a ratoon crop seems highly optimistic. The approach is also being promoted in Pakistan (Hafeez ur Rehman *et al.* 2013).

But beyond ratooning is the development of truly perennial systems of cropping similar to that described for the Apa Tani by von Fürer-Haimendorf long ago. In this endeavour Sacks and his colleagues have been active (Sacks *et al.* 2003a,b) though warning that it is likely to take five to ten years to breed suitable perennial rice varieties for upland areas. Perhaps there are high-production ratooning varieties currently hidden among the very extensive holdings at the IRRI, for that institution has never made a systematic search for them. Given the very large holdings of materials at IRRI that is a significant challenge. A simple start would be to find out if the perennial rice among the Apa Tani still survives and whether there are other communities that use similar cultivars.

In the Association of Southeast Asian Nations (ASEAN) region, which accounts for 22 percent of global consumption, the consumption of rice is driven largely by population growth (Wailes and Chavez, 2012). That has fallen sharply and is now only around 1.1 percent annually. This can probably be met from improved yields, particularly as consumption per person declines, though only slowly at present (Zhang 2007; Wailes and Chavez, 2012). Japan has long seen falling demand for rice though its home production has been artificially sustained by large subsidies. China's demand is also likely to fall. Globally, rice stocks are steadily rising and actual prices show a slight downwards trend, in real terms perhaps more than slight, given rates of inflation in the region. Throughout the region the cheaper grades of rice are already being used as animal feed or in the production of beer. But whatever scientists may think and do, the reality is that the region's increasingly urban people will continue to demand cheap rice, even as the per person consumption falls, possibly at an accelerating rate in future. Ratooning offers a potential to obtain increased production at relatively low cost. That is a bargain to be promoted, but on firm scientific bases.

REFERENCES

Ahmed, T. & Das, G. R. 1988. Scope for rice ratoon cropping in the northeastern hill region of India. In: IRRI, *Rice Ratooning*. IRRI, Los Baños, 119-122.

Apostol, S. 1910, Rice growing in the Philippines, *Philippine Agricultural Review*. 3: 625-638.

Apostol, S. 1915. Dapog method of rice culture, *Philippine Agricultural Review*. 8: 98-102.

Bahar, F.A. 1977. Prospects of increasing tropical rice production through ratooning. *Agronomy Journal*. 69: 536-540.

Baker, J.A. 1940. A Kedah harvesting knife. *Journal of the Malayan Branch, Royal Asiatic Society*. 18(2): 43-45.

Boehm, H-D.V. & Siegert, F. 2001. Ecological impact of the one million hectare rice project in Central Kalimantan, Indonesia, using remote sensing and GIS. Paper presented at the *22nd Asian Conference on Remote Sensing*. 5-9 November 2001, Singapore.

Boyd, V. 2000. *To ratoon or not ratoon*. (Available at www.ricefarming.com/home/2002_MayRatoon.html).

Calendacion, A.N., Garrity, D.P. & Ingram, K.T. 1992. Lock lodging: a new technology for ratoon rice cropping. *Philippines Journal of Crop Science*. 17(1): 1-10.

Chauhan, J.S., Vergara, B.S. & Lopez, F.S.S. 1985. *Rice Ratooning*. International Rice Research Institute. Manila.

Chauhan, J.S, Singh, B.N., Chauhan, V.S. & Sahu, S.P. 1988. Screening of photoinsensitive summer rice (*Oryza sativa* L.) genotypes for ratoon cropping. *Journal of Agronomy and Crop Science*. 160(2): 113-115.

Cheng, M-C. & Li, C-C. 1994. *Inheritance of ratooning ability in rice* (English summary). Research article. 90. Hualien District Agricultural Improvement Station, Hualien.

Clercq, F.S.A. 1871. De voornamste padisoorten geteeld op de droge velden in de Minahasa. *Tijdschrift voor Nederlandsch Indië ser.* 3(5): 1-7.

Colani, M. 1940. Origine et évolution du couteau de moissonneur, In: F.N. Chasen and M.W.F. Tweedie (eds), *Proceedings of the Third Congress of Prehistorians of the Far East,* 1938. Raffles Museum, Singapore, 194-199 and Plates LXIX-LXXIV.

Fageria, N.K. 2007. Yield physiology of rice. *Journal of Plant Nutrition*. 30: 843-879.

FAO. 2013. *July 2013*, Vol. XVI, 3.

Flinn, J.C. & Mercado, M.D. 1988. Economic perspectives on rice ratooning. In: IRRI, *Rice Ratooning*. IRRI, Los Baños, 17-29.

Fukuda, M. 1986. Transition in the traditional rice harvesting system in a Javanese village. In: A. Fujimoto and T. Matsuda (eds). *An economic study of rice farming in Java*. Nodai Research Institute, Agricultural University, Tokyo, 128-139.

Fuller, D.Q., Harvey, E. & Ling, Q. 2007. Presumed domestication? Evidence for wild rice cultivation and domestication in then fifth millennium BC of the Lower Yangtze region. *Antiquity*. 81: 316-331.

Fürer-Haimendorf, C. von. 1946. Agriculture and land tenure among the Apa Tanis. *Man in India*, 26(1): 16-49.

Fürer-Haimendorf, C. von. 1962. *The Apatanis and their neighbours, a primitive society in the Eastern Himalayas*, Routledge and Kegan Paul, London.

Fürer-Haimendorf, C. von. 1966. *Himalayan barbary*, J Murray, London.

Hill, R.D. 2002. *Southeast Asia: People, land and economy*. Allen & Unwin, Crow's Nest, NSW.

Hill, R.D. 2007. *The history of indigenous agriculture in southeast Asia*. (Available at http://library.hku.hk/record=b3121693).

Hill, R.D. 2010. The cultivation of perennial rice, an early phase in Southeast Asian agriculture? *Journal of Historical Geography*. 36: 215-223.

Ho, P-T. 1956-7. Early ripening rice in Chinese history. *Economic History Review*. 9: 200-218.

Ho, P-T. 1969. The loess and the origin of Chinese agriculture. *American Historical Review*. 75: 1-36.

Hunt, C.O. & Rushworth, G. 2005. Cultivation and human impact at 6000 cal yr B.P. in tropical lowland forest at Niah, Sarawak, Malaysian Borneo. *Quaternary Research*. 64: 460-468.

Ichii, M. & Kuwada, H. 1981. Application of ratoon to a test of agronomic characters in rice breeding. I. Variation in ratoon ability and its relation to agronomic characters of mother plant. *Japanese Journal of Breeding*. 31(3): 273-278.

Liu, Z. 2000. Thoughts about the domestication of rice. *Agricultural archaeology*. 2000(1): 122-128. On-line, accessed 30/7/2004 and 29/1/2005. Original in Chinese.

Jones, D.B. 1993. Rice ratoon response to main crop harvest cutting height. *Agronomy Journal*. 85: 1139-1142.

Krishnamurthy, K. 1988. Rice ratooning as an alternative to double cropping in tropical Asia. In: IRRI, *Rice Ratooning*. IRRI, Los Baños, 3-15.

Lamb, A. (Ed). 1961. British Missions to Cochin China: 1778-1822. *Journal of the Malayan Branch Royal Asiatic Society*. 34.

Mahadevappa, M. 1988. Rice ratooning practices in India. In: *IRRI, Rice Ratooning*, IRRI, Los Baños, 69-78.

Matsuo, T. 1997. Origin and differentiation of cultivated rice. In: T. Matsuo, K Hoshikawa *et al.* (Eds), Science of the Rice Plant. *Food and Policy Research Centre*. Tokyo, v.3, 69-88.

Musa, M. 2011. The socioeconomic history of the Orang Kanaq of Johor. *Kajian Malaysia*. 29(1): 47-74.

Neufville, J.B. 1828/1855. On the population and geography of Asam, [sic.] 1828. *Asiatick Researchers*, 16, 331-352. Reprinted in J. Shortt (ed.) *Papers relating to some Tribes on the N E Border of Assam*. T. Jones, *Calcutta Gazette* Office. Calcutta.

Oad, F.C., Cruz, P.S., Memon, N., Oad, N.L. & Hassan, Z.U. 2002. Rice ratooning management. *Pakistan Journal of Applied Sciences*. 2(1): 29-35.

Oad, F.C., Samo, M.A., Hassan, Z.U., Cruz, P.S. & Oad, N.L. 2002/04. Correlation and path analysis of quantitative characters of rice ratoon cultivars and advance lines. *International Journal of Agriculture and Biology*. (Available at www.ijab.org).

Oka, H-I. & Morishima, H. 1997. Wild and cultivated rice. In: T. Matsuo, K Hoshikawa *et al.* (eds), *Science of the rice plant*, Food and Policy Research Centre. Tokyo, 88-111.

Poivre, P. 1770. *Travels of a philosopher, or observations on the manners and arts of various nations in Africa and Asia*. Robert Urie, Glasgow. The 1797 edition is on-line.

Prashar, C.K.R. 1970. Some factors governing rice-ratoon yields. *Plant and Soil*. 32: 540-541.

Rehman, H.U., Farooq, M. & Basra, S.M.A. 2013. *Rice ratooning: a technology to increase production*. Pakissan.com (on-line).

Rejesus, R.M., Samarendu, M. & Balagtas, J.V. 2012. *Forecasting global rice consumption*. (Available at www.agecon.purdue.edu/staff/balagtas/rice_timeseries_v6.pdf).

Rieley, J. 2001. *Kalimantan's peatland disaster*. (Available at www.insideindonesia.org/feature-editions/kalimantans-peatland-disaster).

Sacks, E.J., Roxas, J.P. & Sta. Maria, M.T. 2003a. Developing perennial upland rice I.: field performance of *Oryza sativa/O. rufipogon* F(1), F(4), and BC(1)F(4) progeny. *Crop Science*. 43(1): 120-128.

Sacks, E.J., Roxas, J.P. & Sta. Cruz, M.T. 2003b. Developing perennial upland rice II. Field performance of S1 Families from an intermated *Oryza sativa/O. longistaminata* population. *Crop Science*. 43(1): 129-134.

Sakamoto, S. 1997. *Hsien* rice, *kêng* rice and glutinous rice. In: T. Matsuo, K. Hoshikawa *et al.* (Eds), *Science and the rice plant*. Food and Agriculture Policy Research Center. Tokyo, v.3, 143-153.

Santos, A.B., Fageria, N.K. & Prabhu, A.S. 2003. Rice ratooning management practice for higher yields. *Communications in Soil Science and Plant Analysis*. 34(4&5): 881-918.

Sun Star. 2013. (newspaper) 17 July 2013. 5 000 hectares of rice land in Leyte identified for ratooning. (Available at www.sunstar.com.ph/tacloban/local-news/2012/01/09/5000-hectares-rice-land-leyte-identified-ratooning-199679).

Sweeney, M. & McCouch, S. 2007. The complex history of the domestication of rice. *Annals of Botany*. 100: 951-957.

Sang, T. & Ge, S. 2007. Genetics and phylogenetics of rice domestication. *Current Opinion in Genetics & Development*. 17: 533-538.

Tsunoda, S. 1997. Ecological characteristics of upland rice. In: T Matsuo, K Hoshikawa *et al.* (Eds), *Science and the rice plant*. Food and Agriculture Policy Research Center, Tokyo, v. 3, 135-143.

Turner, F.T. 1993. Rice ratoon crop yield linked to main crop stem carbohydrates. *Crop Science*. 33: 150-153.

Wailes, E.J. & Chavez, E.C. 2012. *ASEAN and global rice situation and outlook*. Asian Development Bank, [Manila].

Watanabe, Y. 1997. Phylogeny and geographical distribution of the genus *Oryza*. In: T Matsuo, K Hoshikawa *et al.* (Eds), *Science and the rice plant*. Food and Agriculture Policy Research Center, Tokyo, v.3, 29-39.

Xu, X-B., Zhang, J-G. & Jiang, X-X. 1988. Ratooning in China. In: *IRRI, Rice Ratooning*. Los Baños, 79-85.

Zhang, W.J. 2007. A forecast analysis on global production of staple crops. (available at www.stats.gov.cn/english/icas/papers/P020071114315023592167.pdf).

27

PRESENT SITUATION CONCERNING THE INTRODUCTION OF PERENNIAL HABIT INTO MOST IMPORTANT ANNUAL CROPS

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There are two main options available for developing perennial crops. The first is through the introduction of perennial traits from wild species into related domesticated crops by crossing or by transferring pertinent genes. The second is through the domestication of wild perennial species using a selection of available biodiversity or through the introduction of domestication characteristics from related domestic species. The first method seems to be the most rapid, while the second could be more difficult and time consuming.

PERENNIAL RICE

Perennial rice is currently the most advanced of the perennial cereal species, as some cultivated rice strains are already able (in humid tropical areas) to have regrowth after crop harvesting. In fact, humid tropical areas could be the first areas to adopt new perennial rice types. In temperate areas the most important limitations for perennial rice may be drought, cold resistance and longevity.

PERENNIAL WHEAT

A high number of progenies derived from crosses of hexaploid and tetraploid (*T. carthlicum*) wheats with several *Thinopyrum* species are available because of crosses made from transferring disease resistance into wheat species. At least a dozen selected perennial wheat lines (out of more than 250 crosses) have now been tested and analysed in international trials. In this material, the main characters to be improved are: shorter straw, earlier ripening, shorter spikes, larger grains, resistance to cold, higher production per hectare, potentiall a smaller number of chromosomes (now most lines are octoploid 2n=56) and chromosome number stability. In the future, some lines could be adopted, especially in polycultures and marginal areas and because of consistent production and cost savings. Some lines could also be useful for dual-purpose grain and forage production.

PERENNIAL RYE

Several selections derived from crosses with the perennial *Secale montanum* are available and adapted to acidic soils and mountain areas, where some rains last the entire year. Further selections should be developed, especially for improved bread making.

PERENNIAL SORGHUM

Several selections of perennial sorghum are now available which are derived from crosses of *S. halepense* (4x) with *S. propinquum* (2x). Some lines of *S. bicolor* are also examples in which regrowth is present. The breeding is looking for both 2n and 4n types. The main limitations are now: small seeds, cold resistance, and shorter straw. The realization of perennial sweet (sucrose) sorghums should also be a priority in order to have the production of seeds, sugar and of straw to be used for animal feed, production of methane or cellulose transformation into sugars. The resilience to drought is an important characteristic of perennial sorghum and its adoption in farming systems affected by climatic events should be further promoted.

PERENNIAL MAIZE

Given the increase in maize seed production obtained in the last 50-60 years, the realization of perennial maize types showing a decent production seems to require several more decades of research, in part because of the very large differences in morpho-physiological characteristics of the perennial related species.

PERENNIAL MILLET

At least two perennial species related to *Pennisetum* are available to transfer perenniality into pearl millet. At the moment there is very little information on breeding for perennial types. Further research and development is essential as perennial millet, sorghum and other drought tolerant crops are key for the food security and livelihood of millions of people in dryland agricultural systems.

PERENNIAL BARLEY

The utilization of *Hordeum bulbosum* for transferring the perennial habit into barley seems difficult because of *bulbosum* chromosome eliminations in F1 crosses. The utilization of other perennial *Hordeum* species should be further explored, especially in lines adapted to marginal areas (e.g. northern, cold climates), requiring short growing cycles.

PERENNIAL OATS

The most likely perennial species present in the *Avena* genus that could be used is the 4n *Avena macrostachya*, found in Algerian mountains and is well suited for areas that require short growubg cycles with limited water. At the moment no information is available concerning this objective.

OTHER SPECIES

Perennial species are also present in *Milium, Panicum, Echinocloa* etc. and related to cultivated ones, which could be used for the introduction of perenniality. Increased policy and research attention should be placed on the wide range of poorly explored and domesticated cereals in order to have the genetic base which allows for a shift towards more sustainable and flexible agricultural systems, enabling farmers to expand their farming options.

GRAIN LEGUMES

At the moment only *Cajanus cajan* is normally used as a perennial grain crop in India and Africa. However, related perennial species are present in *Cicer* (chickpea), *Glycine* (soybean), *Lathyrus, Lupinus, Vigna* etc. which could possibly be used. Grain legumes increase nitrogen availability in soil and are important sources of protein.

SUNFLOWER

There are several perennial *Helianthus* wild species in North America. The introduction of bulbs into *H. annuus* (sunflower) from *H. Maximiliani* (2x) and from *H. tuberosus* (4x) are ongoing, particularly in the United States, with interesting results.

OTHER OIL CROPS

In several annual oil producing species, such as *Carthamus tinctorius*, *Linum usitatissimum* (flax), *Sesamum indicum* (sesame), *Gossypium* (cotton) wild perennial species are present: *Carthamus lanatus, Linum perenne, Sesamum calycinum, Gossypium arboreum* (2x) or *G. barbadense* (4x) etc. that could be used for perenniality transfer.

FORAGE LEGUMES AND GRASSES

Several cultivated forage legume genera (e.g. *Lotus, Coronilla, Onobrychis, Vicia*) perennial related species which could be used to further develop perenniality.

CONCLUSIONS

The objective of introducing perennial traits into many domesticated crop species could interest many breeders working with the most useful species for the improvement of their performance and for saving production costs and labour. Permanent forage species are fundamentally important for improved crop-livestock systems. A wider adoption of diverse perennial forages needs to be further explored by researchers and supported by policy instruments to meet the increasing demand for livestock products and environmental sustainability.

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RECOMMENDATIONS PERENNIAL AGRICULTURE AND LANDSCAPES OF THE FUTURE

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INTRODUCTION

Agriculture has always integrated perennial plants (fruits and forages) and annual crops in different farming systems to enhance diversity and productivity of landscapes while enabling functional ecosystem services and processes to build long-term resilience. But only in the past thirty years have the potential benefits of perennial grain-based cropping systems been recognized as contributing to preventing soil erosion and soil biodiversity degradation, as well as holding the potential to contribute to carbon sequestration. Perennial crops also require reduced amounts of energy, and capture nutrients and water more efficiently relative to their annual counterparts.

As feeding nine billion people in 2050 with increasingly scarce and degraded natural resources is the main challenge faced by humankind, reinvigorating agriculture in a sustainable and productive way on a large scale will take nothing short of a significant shift in agriculture as we know it. With this in mind, there have been a handful of progressive scientists, pioneering practitioners and investors that have been working for over several decades to advance the development of perennial versions of staple crops to be integrated into agricultural systems as a means for operationalizing a true sustainable intensification and the makings of perennial agriculture.

WHAT IS THE CURRENT CONTEXT REGARDING MAJOR STAPLE CROPS?

Globally, there are over 100 million hectares of maize, 240 million hectares of wheat and 158 million hectares of rice. The yields per hectare of these main staples vary widely depending on the presence of abiotic and biotic stresses, inputs and management practices (irrigation, nutrients, pest management, technical support, etc.). And, even though yields have doubled to quadrupled over the past 40 years, these yields have stabilized in the last decade and are further under scrutiny for the concomitant trade-offs in environmental health. It is currently estimated that demands for these products are going to increase dramatically over the coming decades (a doubling in demand for maize is expected by 2050 and a 22 percent increase in demand for rice by 2020) accompanied by increases in demand for inputs (energy, water, fertilizers) if production, consumption and losses systems are not transformed. At the same time, climate change is going to negatively affect yields and reduce the areas conducive to growth. (For example, it is anticipated that maize yields will drop by 10 percent in sub-Saharan Africa and 17 percent in South Asia; wheat yields by 20-25 percent in South Asia; rice will also suffer from yield reductions due to expected water shortages, floods and other extreme weather patterns (Global Futures, 2013). Agricultural systems need to be transformed to be able to address the demand, environmental degradation and issues associated with the impacts of climate change. Perennialized agriculture is an avenue that offers great promise to address some of these issues.

How to get perennial crops?

While historical efforts saw limits in technologies, plant breeding of grains, oilseeds and legumes has undergone a number of advances that promise to make the development of perennial grain crops possible in the next 10 to 20 years. These advances take advantage of traditional breeding techniques such as domestication and wide hybridization to hybridize annuals with perennial relatives in combination with new technologies such as marker assisted selection, genomic in situ hybridization, transgenic technologies and embryo rescue (Glover and Reganold, 2010). Traditional and new technologies are being applied to a host of species including wheat, rice, maize, sorghum, secale, flax, oats, lepidium, camelina, pigeon pea, adlai grass, field pennycress, intermediate wheatgrass and sunflowers - as well as underutilized fruit trees and forages – to serve in new farming systems as perennial food, feed, fibre and fuel crops for the future. In the breeding process, characteristics from wild relatives can be drawn upon to make crops more nutritious, more resistant to pests and with greater adaptive capacity to the impacts of climate change, all of which can increase the capacity of agriculture to address food demands and security.

Progress on all perennial crop species needs to continue, however there are a few systems for which expectations in both the timeline and potential contribution tend to place at priority, including perennial rice systems, dual purpose wheat for grain production and grazing, intercropping perennial legumes and cereals, and boosting of existing perennial systems such as agroforestry and grasslands.

HOW TO FAST TRACK EFFORTS TO TRANSFORM TO A MORE PERENNIAL AGRICULTURE?

The domains that need the greatest attention in the short and long term fall in the categories of research, communications and mainstreaming, enabling policies and public and private investments. While integration among these is needed, the immediate actions needed are articulated by category.

Research

1. **A new generation of breeders and breeding programmes.** Within the context of research, there are a number of tools and assets, include germplasm collections, genomic resources, evolutionary information, cytogenetics and breeding capacity. But above all there is an urgent need for more breeders and breeding of perennial crops, grains and legumes to date, to be adapted to developing country contexts and to investigate new cropping system options. Historically, research in this domain has been more or less supply driven, predominately coupling scientists' interests in a particular crop and the agro-ecosystem of choice. Thus, participatory approaches that fully engage farmers' priorities in diverse contexts must be integrated into the breeding programmes. This can be done through building constituencies and capacities among researchers from Africa, Asia, North America and Europe of relevant disciplines in National Agricultural Research Institutions and programmes and the CGIAR. Simultaneously, farmer-based platforms for assessing, monitoring and promoting practices

can be put in place. Possibly a Centre for Perennial Grain Research could be established allowing for a global collaboration for integration and application of perennials to diverse farming systems and landscapes.

2. **Get the evidence into circulation.** The onus is on the scientific community to provide hard evidence to clearly demonstrate the contribution of perennials to agriculture in order to generate further research investments and farmers communities engagement. There is clearly a need to implement a systematic analysis to screen the highest potential crops, farming systems, and regions and socio-economic contexts in order to achieve short-term goals and early successes for maximum return on investment early on. Field trials and modelling can assist in this prioritization. There is also a need to incorporate robust economic studies to better quantify the overall value of the contribution of perennials.

3. **Breed for innovative farming systems.** There is a need to recognize the short-comings of monocropped farming and embrace efforts to integrate perennials into complex systems including intercropping, rotational cropping, and multi-story cropping systems and integrated crop-livestock-tree systems. Increasing grain production is important, but the added value may be greatest in terms of dual-purpose crops and the co-benefits of perennials for ecosystem services. A coordinated action by the public and private sector, policies, market, and farmers with an integrated effort to assure food security, environmental maintenance and economic returns is fundamental if we have to continue producing food for future generations.

Communications and mainstreaming

1. **Framing the concept.** Language matters in all fields and caution must be taken not to pit annuals against perennials. It is better to frame perennialization as an innovative, complementary and parallel breeding and management effort. That said, it is imperative that perenniality is integrated into mainstream agro-ecological farming and sustainable intensification concepts, and sustainable agriculture and landscape approaches in temperate, humid and dry tropic environments. In this regard, the concepts and benefits of perennial landscapes and perennial agriculture need to be brought more strongly into the conversation as a means to contend with climate change, enhance biological diversity and get back on track to attain safe space in terms of food and environmental security.

2. **Naming new crops.** Some breeders have chosen to provide new names to perennialized annuals as they can be considered new crops. This may be a valuable dimension for markets as well as for increasing the uptake by farmers. Examples include Kernza (perennial wheat) and Montina and Timtana (gluten free Indian rice grass and timothy grass used as grains, respectively).

3. **Actively participating in fora and media.** Each breeding programme needs to emphasize communication and coordination with the global community, taking learning beyond the specific crop dialogues for greater overall learning and benefit. There is a public good on offer that needs to be demonstrated. From the scientific community, communications will be bolstered through key meetings of professional societies (e.g. AAAS, Tri-Societies), dedicated journal issues (e.g. Field Crops Research, Crop Science), and collaborative scientific meetings, particularly held in regions such as Africa and Asia. The Perennial Grain Blog at Michigan State University is a valuable way to share insights among the perennial grain community. (See pwheat.anr.msu.edu/index.php/about/).

Enabling policies

The adoption of perennial crops, agroforestry, and mixed crop/livestock systems to sustain production, food security and rural livelihood, contribute to moving farming systems towards providing multiple economic, environmental and social performance.

Policies promoting this shift of agricultural systems at farm, territory and food chain levels require great commitment and vision coupled with a concrete approach to fit the many local situations. Direct public support (regional and national policies, programmes, subventions, tax, credits) and indirect public support (research, education, development) have contributed in the last sixty years to increase total agricultural production and food chains, but this increase has been obtained with increased energy consumption, Green House Gas emissions, loss of biodiversity, and soils and water degradation.

Renewed policies and programmes need therefore to be developed to reverse this negative trend and also assign a value to public goods such as the maintenance of biodiversity (above and below ground), or the generation of other ecosystem services which are essential to sustain the agriculture of the future.

Some countries have already moved along this direction and developed research programmes adopting a cohesive vision and engaging multiple stakeholders (farmers and their associations, agricultural industry and consumers), schemes to reward production of ecosystem services, land rehabilitation programmes, measures to reduce water and air pollution. Many different labelling schemes have been developed (e.g. organic agriculture, integrated pest management), national programmes to support family farmers, use and maintenance of minor crops, adoption of green technologies and biofertilizers and bio pesticides. Recently some countries have also adopted agroecology laws and are committed to enhance the full potential and diversity of agriculture by combining its economic and social potential while maintaining natural resources.

Hopefully all these programmes and policies will play a catalytic role to promote the shift of agriculture towards securing the food, profitability and ecosystem services that societies want.

Public and private investments

1. **Invest for the long-term outcome**. To develop and scale up the use of perennial grains, oilseeds and legumes take years. Historically, those progressive breeders who undertake these challenges have to do so on the periphery of their other work. Donors need to be willing to invest for the long term with the knowledge that it will be cheaper in many respects than continued short term investments. The recent USAID investment in grain sorghum for sub-Saharan Africa is an excellent example. Farmers and supply-chain companies will need to be sustained in their willingness to engage in testing and adopting innovative farming practices including agroforestry and some of the perennial crops which are in advanced stages for adoption.
2. **Imbed perenniality into programmes and projects**. Both scientists, practitioners, donors, NGOs and other investors have an opportunity to ensure that perenniality gets placed in different programmes and projects that are being designed to enhance progress toward sustainable development goals.

WHAT ARE THE NEXT STEPS FOR FAST-TRACKING PERENNIAL CROPS?

In summary, the integration of perennial species into farming systems, whether crops, forages, or trees can contribute to achieving multiple functions including increased food security and nutrition, climate change resilience and mitigation, increasing energy efficiency and production, and enhancing ecosystems services such as biological diversity, water, nutrients, and land health. In addition, perennial systems can reduce input and labour costs, but many relevant aspects require additional research and extended field tests. Breeding and testing of new management practices will need to provide responses beyond increasing annual yields including evaluation of resistance to cold, dry, humid weather conditions, new pest and weed cycles, soil feedback, and water uptake.

Among the next steps that would be most valuable for enhancing the integration of perennials of all kinds into agriculture, and for fast-tracking the development of perennial grains, oilseeds and legumes forward would include key investments in:

- Ramping up research to advance promising perennialized species, ensuring a global network that is addressing demand and co-research and learning with farmers' platforms in the context of developed, emerging and developing country contexts;
- Ensuring cross learning and collaboration among scientists globally working on various species and hosting workshops and conferences in key regions and countries (e.g. East, West and Southern Africa, China, Brazil);
- Enhancing communications of the evidence of perennialized species' contributions to addressing local and global development challenges;

- Mainstreaming the concept of perennial agriculture into research, practice and national, regional and global policy and investment fora as well as through a variety of communications and social media;
- Identifying a small team to articulate the specific architecture and costs of a virtual and ultimately bricks and mortar Centre for Perennial Grains Research or Centre for Perennial Agriculture;
- Articulating and developing an impact pathway for achieving a global target of hectares of annual-based agriculture transitioned to perennial agriculture in a diverse set of countries.

REFERENCES

Global Futures. 2013. *A CGIAR Collaboration*. (Available at http://globalfuturesproject.org).

Glover, J.D. & Reganold, J.P. 2010. Perennial grains: Food security for the future. *Issues in Science and Technology*. Winter 2010: 41-47.

THE WORKSHOP

Based on this background, and recognizing that "hard data" and successes need to be published, FAO, in collaboration with the Consiglio per la Ricerca e la sperimentazione in Agricoltura (CRA), The Land Institute, Charles Sturt University, CSIRO and other related institutes, hosted an Expert Workshop on Perennial crops for Food Security, from 28th to 30th August 2013, in Rome.

The workshop brought together senior level professionals, policy makers, top level breeders, geneticists, soil scientists, social scientists and NGO's to take stock of the best information available today on perennial crops and to discuss new cropping systems and rotations for different ecologies to create awareness on their potential for improving food security and increasing climate change resilience, efficiency, ecosystem management, and creating new economic opportunities.

Further information and video interviews with the authors can be found at the following sites:

- **YouTube videos**
  www.youtube.com/playlist?list=PLzp5NgJ2-dK4_itTMZqwUEg4BTBymkWgw
- **FAO**
  www.fao.org/agriculture/crops/thematic-sitemap/theme/spi/fao-expert-workshop-on-perennial-crops-for-food-security/en/
- **Land Institute**
  www.landinstitute.org/vnews/display.v
- **Michigan State University**
  http://pwheat.anr.msu.edu/index.php/tag/sieg-snapp/
- **Charles Sturt University**
  www.csu.edu.au/research/grahamcentre/
- **CRA**
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PERENNIAL CROPS FOR FOOD SECURITY
PROCEEDINGS OF THE FAO EXPERT WORKSHOP

This publication presents the latest research in perennial crop breeding and programmes, and provides direction on where the field of perennial crop is heading. Many production systems and agricultural practices are no longer sustainable today as their effects on soils, water, biodiversity, and livelihood are significant. Mainstreaming the use of perennial crops into current practices can contribute to stabilize fragile soils and maintain natural processes essential to obtain stable and high yields. To face the challenges and risks of the twenty-first century, increasing the perenniality of crops and agricultural systems should become a larger research, development and policy focus.

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