10

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

Mulatu Geleta[1], Li-Hua Zhu[1], Sten Stymne[1], Anna Lehrman[2], Sven Ove Hansson[3]

1 Department of Plant Breeding, Swedish University of Agricultural Sciences, P.O. Box 101, 230 53 Alnarp, Sweden

2 Department of Crop Production Ecology, Swedish University of Agricultural Sciences, P.O. Box 7043, 750 07 Uppsala, Sweden

3 Division of Philosophy, Royal Institute of Technology, Teknikringen 78, 100 44 Stockholm, Sweden

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.