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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
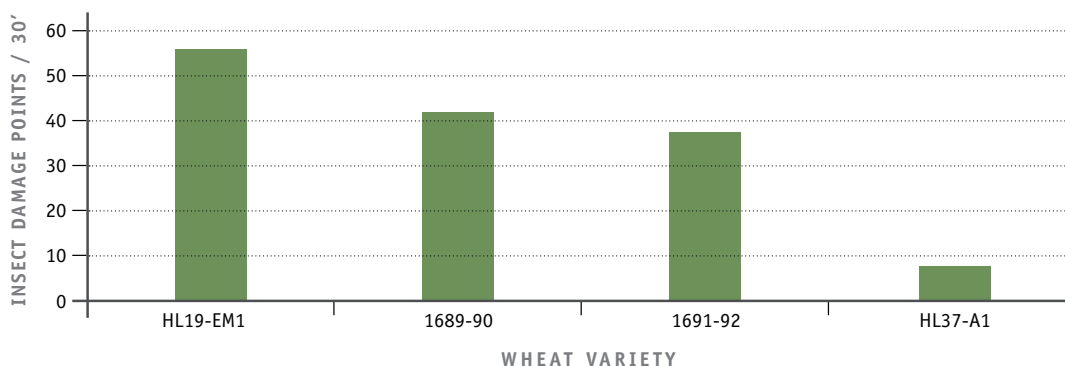
Source: Morris *et al.* 2006

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.

ACKNOWLEDGEMENTS

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.