Final Report to the Ceres Trust.

Project Title: Breeding and testing corn for organic farmers that combines high N efficiency, superior nutritional value, and cross incompatibility.

2015 to 2017

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Introduction

Corn presently produces more grain than any other single cereal in the World. It is also the major cereal crop for both conventional and organic farmers in the Midwest (Anon. 2016). Because of consolidation of the seed industry and the predominance of transgenic, genetically engineered (GE) corn, the diversity of elite corn cultivars for organic seed companies and farmers in the USA has decreased. GE contamination has become widespread and has polluted the entire food chain. At the same time, the nutritional value of the grain has decreased because commercial breeding focused mainly on higher yields.

Organic farmers need corn varieties that are well-adapted to organic conditions, produce high yields, and provide better nutritional quality and ecological services. That corn should be easy to grow without a lot of inputs, be efficient at obtaining nitrogen (N) and other nutrients from soil with little fertilization. It should be free of GE contamination and biologically cross incompatible with GE corn.

The Mandaamin Institute has been developing varieties to combine these characteristics. The results should refine and re-define what corn is for organic farmers.

The grain of the Mandaamin varieties possess higher protein, protein quality, and carotenoid content than conventionally bred corn. Therefore, those varieties should meet the methionine needs of organic dairy and poultry producers and set a new standard for egg yolk and meat color in poultry.

The Institute selected corn to grow well under N limited conditions by being better at extracting N from soil organic matter. But it also bred corn that can interact with bacteria and obtain N from soil or N_2 from air. Nitrogen efficiency should reduce the need for nitrogenous fertilizers for organic and conventional farmers.

The Institute is also developing cultivars with naturally occurring genetic systems that prevent pollination from GE corn by not permitting pollen tube growth on silks.

Our big challenge has been combining these traits and bringing yield levels up so that the cultivars that have them are competitive. Adequate yield is necessary if seed companies will offer them and farmers will grow them. The Mandaamin Institute is a small non-governmental organization with a devoted team that are effectively tackling this problem. Therefore, we greatly appreciate the financial assistance from the Ceres Trust. That help has enabled considerable progress in breeding the kind of corn the World needs for positive change.

The new website from the Ceres Trust shows us true depth of interest and understanding of our current world situation and the direction that help must come from. In light of that information, we will depart from the normal, short, factual report of results to give a more in-depth explanation of what we have been doing with funding from the Ceres Trust and others. This report to the Ceres Trust will describe the larger-scope background of the problems that we are tackling, why they are important, and then our approach to resolving the problems. After providing this context, the work we did with the financial help of the Ceres Trust and other organizations will be described. We will interpret and discuss our results and our progress, and outline future steps that need to be taken to bring the work forward.

Context

N fertilizer and synthetic methionine are inputs used to assure adequate corn and animal production and thereby sustain corn markets and farmer profitability. Genetically engineered traits are regarded as convenient for conventional farmers and profitable for large corporations. These inputs support and sustain conventional farming systems and farmers. But there are major problems associated with conventional farming systems and the widespread use of these inputs. This industrialized approach feeds masses of people. But it also results in dead soil, pollution of water, soil, and air, global warming, poorer quality food, chronic illness, and dislocation of farmers and farm animals from the land.

Before digging into these problems and how we think they can be resolved, it is important to realize that those problems grow out of the predominantly materialistic and pragmatic way our culture thinks about the World. Corn-rich rotations, synthetic N fertilizer, synthetic methionine, and transgenic crops are simply efficient shortcuts to food production. They may be profitable in the short term. The predominant mindset views soils, plants, animals as biological machinery, and farm methods as input-output issues. Decisions are based on optimizing profitability. Farmers are compelled to follow the dictates of efficiency and profitability in-so-far as possible and not the dictates of good stewardship of soil and domesticates.

A reductionist mindset also pertains to breeding crops. Plants are viewed as collections of genes. Breeding is viewed mainly as efficiently collecting good genes and getting rid of bad ones. Breeding corn has mainly become a factory-like process carried out using the shortcut of di-haploid technology. Products are 'improved' with artificial, novel, patented, transgenic gene 'traits' that ensure profits for corporations and stockholders. The profession of being a plant breeder used to entail conducting, overseeing, and being responsible for a whole program that improved whole plants or animals. That profession is disappearing. Plant breeder functions are being subsumed by lab scientists who do molecular breeding. They no longer take cues and actions based on observing whole plant performance in the fields. Therefore, a 'feeling for the

Organism' (Keller, 1983) and a sense of biological balance and integrity for the whole organism does not have the opportunity to grow in them.

So how is it possible for us to change our approach? How can we find and develop more natural, holistic, and synergistic approaches that are financially profitable, health giving and productive for our co-creatures, consumers, and farmers? And how do we develop a holistic approach to breeding crops that educates breeders and will result in varieties that will help farmers and engender healthier agriculture and consumers?

Mandaamin Institute's breeding approach: Native people bred corn, creating a cob from a tassel on side branches of the wild grass teosinte and shifting the sexuality of the flowers from male to female. That remains an unprecedented accomplishment that is presently taken as a given. In the 1980's, when I began working with native corn as well as corn belt dent corn, I became interested in better understanding the contrast between the way modern culture treats corn and the understanding of the native cultures out of which it had sprung. In search of understanding I had, and continue to have, conversations, especially with Hopi, Oneida, Arikara, Cherokee, Ho Chunk, and Haudenosaunee people that actively grow corn. These conversations have made me aware of just how different the native perspective of corn is; so different to our present culture that it is considered to be out-of-bounds for conventional thinking. For thousands of years corn has been the most holy crop and food of many of the native tribes that lived in the Americas before the European invasion. The stories of the Zuni people documented by Frank Cushing in the late 1800's (Cushing and Wright, 1992), and the traditions of many of the Pueblo peoples, suggest that the creation of this crop was a long-term project directed out of mystery cults associated with different clans. Corn is viewed as a gift developed in cooperation with the Spiritual world; as another aspect of being human; as a bridge to the dead; the dead participate in growing corn from the other side of reality; as being like a relative (see also Prechtel, 2012, for a Mayan perspective).

Conversations I have had with Hopi elders and with Carl Barnes (Cherokee), threw light on the native approach to evolving (breeding) corn. It differs significantly from how breeding is generally done and regarded today. According to my understanding of those conversations, the spiritual activity of the person working with corn was regarded as critical as are the environmental conditions in which the corn is grown. The plant is a living entity that is connected with the inner life of people. The corn is regarded as being capable of 'remembering/expressing' buried traits under the right circumstances. The corn is connected with families and cultures. Evolution occurs as a kind of dialogue between human activity and plant creativity, and the times, environmental changes, and human attitude, observation and selection are critical elements.

Plants and people are both active partners in the evolutionary process, playing important roles. This relationship contrasts ultimately to the view of corn in our present culture as being genetic machinery/commodity/plant material that can be manipulated at will by individuals and corporations for profit. The consequences of learning from the native approach are the arrival of open questions. Does co-evolution means that transformation in the corn should be paralleled by transformation in the breeder? Is it the task of the breeder to strive not only to achieve breeding objectives but also to changes in attitude; beyond an I-it, relationship to an I-Thou relationship, in the sense of the philosopher Martin Buber?

The forming of the Mandaamin Institute in 2011 allowed full focus on the problem of fostering corn and transforming it and our culture's relationship to it. The name Mandaamin is famous from Longfellow's poem (the Song of Hiawatha; Longfellow, 1855) as the spirit of corn that had to be wrestled with and whose sacrifice becomes our food. That story is grounded in Anishinabe myth (Johnston, 1976). Mandaamin is an Algonquian word that means corn or more literally 'wonder seed.'

Choosing this name for our Institute meant choosing to attempt to bridge the cultural divide and combine the gifts of the plant that have been achieved by native and scientific breeding into a new, higher synthesis.

That is of course, a personal challenge, but how is it to be done? To begin with, it may be important to realize that breeding actually is a reflection not only of the plant and its genome, but also of human culture. Breeding is also a process in which humans can become inextricably *inside*, involved in a partnership with a plant. It is a dynamic process that integrates cultural values and technology, breeder attitudes, experience, observations, skills, and actions, specific selection environments, and plant whole-body responses and adaptations.

Secondly, it may be important to develop a respectful attitude, different ways of knowing agriculture that are more qualitative, and to implement healthy, living farming systems. The two go together. Just as conventional reductionist thinking results in conventional farming, so does integrative thinking result in organic/biodynamic farming, as it did with native farming. Conventional and organic kinds of farming have different needs. In any case, it makes sense to select varieties under the conditions which they will be grown in order to increase adaptation. Hybrids developed under conventional conditions can produce high yields under organic conditions. But aside from yield, corn for biodynamic/organic farms needs traits that conventional corn does not possess. Conditions on biodynamic and organic farms are such that the varieties should be able to obtain sufficient nutrients including nitrogen without synthetic fertilizers, compete well with weeds without herbicides, and meet the nutritional needs of farm animals. Plants must reliably demonstrate a high level of quality and yield under stress and non-stress conditions.

At the Mandaamin Institute we have tried to cultivate a different attitude towards breeding. Our perspective is that corn is a flexible, biologically creative, and only partially understood ***partner*** to interact with. We exercise respect for the integrity of the crop by using classical breeding methods. We select on farms that use biodynamic and organic practices using criteria that address the performance of the whole plant. This 'partnership-with-a-plant' in a learning/'dialogue' mode is a significantly different standpoint of departure from the 'manipulate-the-genetic-mechanism' approach of conventional breeding. It adds to it systematic attentiveness to the whole plant in the field, including to (epigenetic) adaptive efforts by the plants. Our approach is also grounded in the phenomenological scientific method promoted by Johann v. Goethe, Rudolf Steiner, and others (Holdrege, 2013). This approach is in consonance with biodynamic, organic, and native farming philosophy which works from the whole to the part and views the human role as shifting to develop respect, partnership, and stewardship with domesticates.

N fertilizer problems: Nitrogen fertilizer is big business in the US and globally. The amount of N fertilizer applied to corn in the US increased from 1.6 million tons of actual N in 1964 to 5.6 million tons of N in 2010 (54) with 82% of it coming from other countries (main producers China, Saudi Arabia, Kuwait, Quatar) (Kelischek, 2012). Production of N fertilizer accounts for half of the energy needed to produce and dry corn (Hanna and Sawyer, 2012). Manufacturing the N used to grow one acre of corn in Iowa is equivalent to the energy in 26 gallons of diesel fuel (Hanna et al., 2010; Plastina, 2017). In general, price of fertilizer has increased relative to the value of crops, over time (USDA-ERS. 2012). The price of anhydrous ammonia N-fertilizer is tied more to the price of a bushel of corn (what the market will bear) than to the price of natural gas that is used to produce it (Schnitkey, 2016). But N fertilizer accounts for about a quarter of farmer-bought inputs for field production (Plastina, 2017, USDA-ERS, 2016), and as many farmers will be losing money at current grain prices, reducing N fertilizer inputs is of interest for conventional farmers.

N fertilizer not only secures grain yields, it also causes nitrate pollution of water (Nolan et al, 1998 and 2002) which causes human cancer, less fertility, blue-baby syndrome (Townsend et al., 2003), and increased expenses for producing unpolluted water for consumers (MN Dept. Ag., 2017; EPA, 2015; Eller, 2017). It causes a growing hypoxic dead zone in the Gulf of Mexico (Dodds, 2016; Scavia et al., 2017; NPR, 2017). The dead zone decimates the shrimp production industry (Smith, et al., 2017), and raises prices for consumers. Also, N polluted surface waters increase problems with algal blooms that produce toxins that poison water supplies (Pelley, 2016) and foster mosquitoes that carry infectious diseases such as West Nile Disease (Townsend et al, 2003). N fertilizer increases nitrous oxide pollution (Park et al., 2012) which causes global warming, reduction of ozone in the upper atmosphere, and greater problems with asthma and reduced resistance to infectious diseases (Townsend et al., 2003).

Nitrate pollution of well water from fertilizer has become a serious national health and financial problem. Municipalities are drilling new wells, blending waters, or installing nitrate removal systems (MN Dept. of Ag., 2017). Costs to municipalities for producing potable water from N polluted water are 5 to 6 times more expensive than for water with low levels of nitrate (EPA, 2015). Expenses for small communities are higher. Costs of installation or drilling new wells in municipalities in Minnesota range from $350 to $1,000 per resident; and the ongoing cost of drinking water has increased by a factor of four for nitrate contaminated water (MN Dept. of Ag, 2017). The Water Works of DesMoines, Iowa sued three counties (Eller, 2017) for polluting drinking water for ½ million people in Iowa. They estimated that it would cost them $80M to get equipment to remove nitrate and had cost $1.5M to do it the previous year. Clearly, restrictions on N fertilizer use need to be conceived of and implemented. Nitrogen cap and trade programs are already in place or being implemented in the Chesapeake Bay Area (http://www.mdnutrienttrading.com/ntwhatis.php), and in the Ohio River Basin (http://wqt.epri.com/).. We assume this trend will continue into the accelerating less N fertilizer use and more sustainable or organic farming practices.

However, fertilizing and pollution are not only an issue for conventional farmers. Our experience in discussions with organic farmers is that they can over-fertilize corn with manure. Corn is viewed as a heavy feeder and manure resources are directed to it. Farmer thinking follows a simple input-output model for nutrients that does not apply to the reality of the organic farm soil. Over-applications of manure (especially chicken manure) may be a kind of insurance for a good crop, but they are pollutive. Fortunately, application rates are partially tempered by the high cost of these inputs.

Mandaamin's approach to resolving N fertilizer problems. In contrast to the idea that corn is a heavy feeder that needs lots of N, our three year surveys of organic and conventional farmers in Wisconsin, Illinois, and Iowa found that corn is a N efficient crop that may or may not respond to fertilization (Goldstein and Cambardella, 2008). Corn seemed capable of obtaining N from both poor and rich soils in similar quantities. In fact, on poor soils, more N was taken up than was expected on the basis of measured spring nitrate N and predicted rates of N release from soil organic matter. A major problem for the Midwest seemed to be that for economic reasons, corn is rarely rotated with perennial grass/legume leys. Too often corn follows corn in rotation, resulting in increases in root disease and consequent need for soluble N fertilizers (Goldstein, 2001; Goldstein and Cambardella, 2008).

A decade ago I did (unpublished) research with dairy farmer Walter Moora to determine the best place to put manure on an organic/biodynamic farm. We discovered that manure resources might be better applied to grass/legume forages than to corn. This might result in greater productivity, less nitrate pollution, and far greater carbon retention in the soil. This idea finds confirmation in the scientific literature. For example, the long-term Sanborn Plot experiments in Missouri showed that the best way to multiply soil organic matter was to apply manure to grass (Miles and Brown, 2011).

On the other hand, N efficiency in corn can be enhanced through breeding and selection under organic conditions (Goldstein et al., 2010). Sustained breeding for yield or N-efficiency may not only increase the reproductive efficiency of the plant. It also increases root production and the ability of corn roots to extract N from soil organic matter (Ma et al., 2001, Edmeades et al, 1996; Lafitte and Baenziger, 1997; Kamara et al., 2001).

Years ago our breeding team began breeding for N-efficiency by growing corn on N-limited, organically managed soils and selecting for corn grain yield and methionine. In 2009 we grew our resulting inbred lines alongside conventionally-bred inbreds on a N-limited field. We measured chlorophyll in leaves and compared the isotope composition of grain and tops using the natural abundance method (Boddey et al., 2001). This method uses N isotope ratios to gain insight into whether N is coming primarily from soil (richer in ^{15}N) organic matter or from air (richer in ^{14}N). We found that our breeding lines were darker green, had greater contents of chlorophyll, and had higher delta ^{15}N isotope ratio in the grain and tops than conventionally bred inbreds (see Goldstein, (2017), p. 12). This meant that they had probably mineralized more N from soil organic matter.

Another route to N efficiency is to increase the ability of corn to enhance N_2 fixation from the air by microbes that live in or alongside the plant. Researchers have confirmed that certain landraces of corn foster N_2 fixing bacterial colonies in their tissues and in the mucilage on their brace roots (Schwartz, 2009; Zamora, 2017). These bacteria fix N_2 from atmospheric gas. The N that is fixed is then taken up by the corn plant and turned into plant protein (Goldstein, 2016).

Our breeding program bred for N efficiency/N_2 fixation. We crossed Corn Belt inbreds with landraces that we think are fixing N_2 with the help of bacteria. We think they are fixing N_2 because they respond to inoculation with N_2 fixing (diazotrophic) bacteria, perform relatively well on N limited soils (stronger growth, more N-rich chlorophyll), and show shifts in their natural isotopes in favor of ^{14}N that indicate that up to half of their N may have come from air (Goldstein, 2016). Several of the upland landraces from Mexico appear to be efficient, and the trait may be present in many other Mexican landraces that we have not tested. We believe we have also found the efficiency trait in both lowland and highland races from South America, indicating that the trait may be widespread having been selected for under conditions of native farming.

Synthetic methionine: Synthetic methionine has widespread use in both conventional and organic poultry farming. Even organic farmers are permitted to feed synthetic methionine to chickens in restricted amounts. The use of synthetic methionine in poultry causes them to produce more of the growth hormone IGF1 (Carew et al, 2003; Del Vesco et al., 2013; Wen et al, 2013) which aids in production of meat and eggs. But consumption of food rich in IGF1 has been implicated in increased incidence of human cancer (Kaaks, 2004). In any case feeding synthetic amino acids is not congruent with what many organic consumers expect in organic eggs or chicken meat. In 2016 the Organic Consumers Association presented a petition to the National Organic Standards Board to forbid the use of synthetic methionine with 14,000 signatures due to the linkage with IGF1. The USDA has stated that they will phase out its use (McEvoy, 2015).

The larger organic poultry industry has lobbied to retain use of synthetic methionine (Brunquell, 2017). It is argued by industry that methionine is an essential amino acid and it does not matter if it is made available to poultry in a natural or synthetic form. Closer inspection shows that synthetic DL methionine, which is probably the synthetic form that is mostly fed to poultry, is a 50:50 mixture of two chiral molecules, D-methionine and L- methionine (Goodson, et al., 2012). D-methionine is rarely ever found in natural systems. Once across the intestinal barrier, chickens convert the D-methionine to L-methionine enzymatically in their liver or kidney and then use it (Goodson et al., 2012). Though the natural and synthetic substances induce different activities in the body of the bird, we lack information on the consequences. Pertinent to the knowledge gap is that different forms of synthetic methionine may differ, at times strongly, in their effects on increasing IGF-1 levels (Del Vesco et al, 2013; Willemsen et al., 2011) in poultry.

Breeding high methionine corn: In earlier work we bred high methionine, lysine, and cysteine corn based on utilizing the floury-2 (fl_2) allele. Initial feeding trials (Organic Valley and University of Minnesota) suggested that high methionine fl_2 corn (approximately 0.3% methionine) might replace synthetic methionine for organic poultry (Jacob et al, 2008; Levendoski and Goldstein, 2006), confirming earlier studies (Cromwell et al, 1968; Chi and Speers, 1973). Birds produced similar egg or meat production to conventionally bred organic corn + synthetic methionine. However, the birds fed our corn exhibited different behavior and biology (greater enthusiasm for the feed, no cannibalism, less jumbo eggs).

In order to breed for high methionine corn it was first necessary to develop methods for quickly, and cheaply measuring it. In conjunction with Iowa State University cooperator Charles Hurburgh, using Near Infrared technology, we broke the inherent correlation between protein content and methionine, lysine, and cysteine content for the first time (Jaradat and Goldstein, 2013 and 2014). These three essential amino acids are critical for human and animal nutrition. The natural correlation was broken by constructing unique sets of corn grain calibration samples from our breeding program, containing different levels of these amino acids at variable levels of protein. Using the calibration based on this technology with over 400 wet chemistry analyses we were able to breed productive inbreds and hybrids with more methionine in their grain and softer, more digestible kernels than conventional hybrids. The correlations between NIRS and wet chemistry results have been highly significant on all years tested, giving us a cheap and accurate method for testing for these amino acids.

However, the grain yields of our fl_2 corn were inadequate, in part due smaller seed size, (see also Lorenzoni et al, 1980; Coleman et al., 1995). Therefore, we screened our ***non*** fl_2, hard endosperm breeding families for high protein, methionine, cysteine, and lysine. We found a new set of opaque kernelled selections in a wide set of 13 breeding families, mostly derived from crosses of normal, translucent-kernelled landraces with normal commercial inbreds. These kernels had little or no reduction in seed size and yield and had high nutritional quality (Jaradat and Goldstein, 2013, 2014, 2018 and Goldstein, 2012a). Subsequently, there has been continued spontaneous occurrence of opaque kernels in populations, breeding lines, and in inbreds, which we have utilized in our breeding program. Crossing tests and kernel weight differences between translucent and opaque kernels showed that the new trait is not fl_2.

In 2011 wet chemistry results showed that six of our new floury hybrids averaged 10.9% protein, 0.25% methionine, while eight conventional hybrids which had been grown next to them averaged 8.1% protein and 0.17% methionine. Qualitative gel electrophoresis (conducted by Dr. David Holding, University of Nebraska) showed that the types of protein in the grain of our breeding lines had shifted with less α zeins that have poor nutritional value and more β and δ zeins and non-zein proteins that have enhanced nutritional value.

Cross incompatibility.

The genes Ga1, Ga2, and Tcb1 are found in teosinte (wild corn) and in landraces of sweet corn, field corn, and popcorn from Central America. These genes convey a trait called gametophytic incompatibility or cross incompatibility. The presence of these alleles, especially in the homozygous state, prevents pollination by corn pollen that does not possess the same alleles. Thus if corn has these genes they can avoid contamination by GE corn. The Mandaamin cross incompatible corn was bred by crossing our corn inbreds with corn stocks possessing these traits. The Ga1 trait was obtained from popcorn. It has been transferred to white corn varieties. We obtained seed with Ga1 from Missouri and in conjunction with Iowa State University and USDA-ARS (Moises Gonzales, Linda Pollak, Paul Scott), we backcrossed the trait into a set commercial-grade inbreds. We used these inbreds with the trait as breeding stock sources for the trait and then backcrossed it into our high methionine, N efficient corn.

Photo 1. Tropical corns being used to transfer traits to our breeding lines in Puerto Rico:

Left. Amazonian corn with multi-aleurone trait associated with more minerals, B vitamins, and higher protein quality.
Center. Mexican corn with multiple genes for cross incompatibility (Ga1s, Tcb1, Ga2s).
Right. Mexican corn with demonstrated ability to fix N in conjunction with endophytic bacteria.

Project Objectives.

The project funded by Ceres Trust and others enabled Mandaamin Institute to pursue the following objectives: 1) through breeding and selection to combine N efficiency/N_2 fixation, nutritional quality, and grain yield into elite inbreds and hybrids, 2) to test those hybrids on organic farms with different levels of fertility; 3) to identify which of these cultivars has potential for improving corn production for organic farmers; 4) to communicate results to farmers, companies, and scientists through field days, presentations, and publications.

Project Structure:

The Ceres Trust funding was a three-year project. Funding from Ceres Trust covered some of the expenses associated with the ongoing project in Wisconsin and supplemented funds we obtained from a NIFA-OREI grant. The plan outlined in the initial proposal to the Trust was carried through without essential changes. The project involved producing and testing hybrids made with our cultivars for their yield in replicated trials on high and low fertility sites. It also involved nurseries to combine the traits into inbreds and to select the best under N limited conditions.

Methods

Mandaamin Institute uses a standard inbreeding-based selection program coupled with early generation testing of grain yields. Occasionally, inbreds or hybrids were used to make improved synthetic populations, from which subsequent inbreeding and selection occurred. Breeding took place on nearby organic farms. Mandaamin systematically tested inbreds for N efficiency on field sites that are low in available N. Organic winter nurseries at the University of Puerto Rico (Lajas) and with Hawaiian grower Marie Mauger (Anahola) also enabled Mandaamin to have two breeding or multiplication seasons each year.

Selection and inbreeding: Selection for N efficiency/N fixation of breeding lines occurred in nurseries under N limiting conditions. Plants are grown on soils with sub-optimal organic matter content (Rohrer, 2015-2017), or under N immobilizing conditions caused by grass thatch buildup. Corn was also grown after a preceding crop of corn (Goldstein, 2015, Little Prairie, 2015).

Inbred lines grown under these conditions were evaluated in early June for emergence, color, and height. Values for each of these parameters are converted into a fraction of the mean overall value, which are averaged for a given breeding line. With some exceptions, only lines that are above the overall mean were self-pollinated and lines with unusual disease or insect infestations were not.

Nitrogen efficiency, N fixation: Seed was inoculated with a mixture of diazotrophic bacteria that have been isolated from the roots, rhizosphere, rhizoplane, and internal tissues of corn. This inoculate consists of a unique consortium of 8 N_2 fixing bacteria that live in different parts of the plant (around the roots, on the roots, inside the plant). These strains were selected in previous studies based on their impact in combinations on susceptible plants in the field. The inoculate increased N efficiency, N uptake and N_2 fixation as measured by natural isotope abundance, chlorophyll scores, root /microbe dynamics and N uptake by the plants. This consortium of microbes was derived by Walter Goldstein in conjunction with Terra-Max, Inc.(Bloomington, MN), and it was formulated for use in research by Terra-Max.

Yield trials: Crosses for hybrid yield trials were generally made starting at the S2 or S3 stage of inbreeding by crossing breeding lines with inbred testers from an opposite heterotic group.

Replicated yield trials of up to 1000 hybrid entries took place on the Zinniker, Rohrer, and Goldstein farms. The Zinniker Farm was considered to be a high fertility site as corn followed alfalfa on a medium textured soil with a high level of fertilization (estimated at 40-60 tons of cattle manure/acre). On the Goldstein Farm corn followed alfalfa grass or corn. The Rohrer Farm yield trials were regarded as nutrient limited as corn followed rye with a low (2-3 ton/acre) level of fertilization with composted chicken manure. Plot size on all sites was 5 feet x 17.5 feet. Where there was sufficient hybrid seed, replicated plots were grown on both high and low fertility sites (2016). The maximum number of replicates on each site was 3. Plot yields and grain moisture content were determined at harvest with a Gleaner combine modified to weigh plot yields and with a portable moisture meter. At harvest a grain sample was taken from each plot and protein, oil, methionine, lysine, and cysteine content of whole grain were determined by a a near infra-red spectroscopic (NIRS) calibration (Jaradat and Goldstein, 2013). Grain and protein yield, methionine and lysine content, and moisture data obtained from these trials are decisive for determining the inbreds to continue to inbreed. The selection pressure generally ranging from 2 to 15% depending on the results.

Cross Incompatibility.

We continued to backcross the Ga1 gene (derived from popcorn) into our breeding lines while systematically testing for the active presence of the gene. The source for this trait was a synthetic made by crossing white corn inbreds with the trait (Mo508 x Mo506).

Combining Ga1 with Tcb1 might provide a stronger barrier than just using Ga1. Therefore, Maiz dulce, a Mexican sweetcorn landrace, was used as a second source for our breeding because it combines the Tcb1 and Ga1 genes. This sweetcorn was crossed with the old Pioneer inbred PHZ51 by Major Goodman, corn breeder at North Carolina State University, and the traits were stabilized into a homozygous condition. Dr. Goodman used pollen from the stabilized version to cross with our breeding lines or inbreds, and we subsequently backcrossed the trait into those same lines or related lines.

In the breeding process we developed populations that segregated for the presence or absence of the genes. To determine whether a plant had the genes in an active state, we used two methods. In the first method, we self-pollinated the plants but also used pollen from plants to cross with a 'tester' plant that we knew had the Ga1 genes in a homozygous state. If the plant in question had at least one allele for Ga1 it would make a cross with the tester plant. Otherwise the tester plant would be barren.

The second method was to take pollen from a plant that had blue aleurone kernels (Specht Blue, bred by Dan Specht, deceased cooperating organic farmer from Iowa). That pollen was applied to the silks of the plant in question first. Then, afterwards, we pollinated the plant with approximately the same amount of pollen from itself. If the resulting ear had no or less than 7 blue kernels, we kept it for further breeding If there were no active Ga1 or Tcb1 alleles in the plant the plant would have many blue kernels.

Farmer and Business Participation: The success of our undertaking depended on the goodwill of farmer and business cooperators. Indeed, the outpouring of support from them was remarkable and indispensable. The actual crossing/selection and overall breeding work was generally carried out by the Mandaamin team. However, farmers participated by providing land, tillage, fertilization, viewing results and providing advice on how to proceed. In some cases land use was donated in exchange for extra grain, in other cases land was rented at a reasonable price. Valuable advice was given by participating farmers and farmers who came to our annual field days. The breakdown of acreage by farm and use, and field day sites are shown in Diagram 1.

Diagram 1. Acreage on different organic farms and field day sites.

	2015					
Farm/Farmer	yield trials	nursery	seed mult.	hybrid prodn.	Total	field day
	acres planted					
Rohrer	5				5	x
Zinniker	2				2	x
Goldstein	1	1			2	x
Maikop		4			4	
Peterson	0.5		1		1.5	
Amish Farmer	0.1				0.1	
University of PR		0.5			0.5	
Total	8.6	5.5	1	0	15.1	

	2016					
Farm/Farmer	yield trials	nursery	seed mult.	hybrid prodn.	Total	field day
	acres planted					
Rohrer	5	4	2		11	x
Zinniker	3				3	x
Turtle Creek				2	2	
Goldstein				0.34	0.34	
Amish Farmer	0.1				0.1	
University of PR		0.5			0.5	
Total	8.1	4.5	2	2.34	16.94	

	2017					
Farm/Farmer	yield trials	nursery	seed mult.	hybrid prodn.	Total	field day
	acres planted					
Rohrer	3.6	2.4			6	
Zinniker	3				3	x
Mantoan			0.75	0.75	1.5	
Goldstein			0.1		0.1	

Amish Farmers	0.1		0.2		0.3	
University of PR		0.5			0.5	
Mauger			0.1			
Total	6.7	2.9	1.15	0.75	11.5	

The greatest amount of land utilized was on the farms of Rohrer Enterprises in Elkhorn and East Troy, WI (Jim Rohrer). Jim Rohrer did tillage work on fields, paid for cover crop seed, and also provided his organic café in East Troy, Wisconsin as a meeting place for discussions for the first field day. We were fortunate to be able to grow yield trials on the Zinniker Family Farm in Elkhorn (Mark and Petra Zinniker) which is the oldest biodynamic farm in North America. Furthermore, we had test trials on the Goldstein Farm in Elkhorn, on the Maikop farm in Little Prairie, and on the Yggdrasil Nokomis Farm under organic farmer Joe Mantoan. The first year of the project John Peterson helped us to multiply one of our best inbreds on his vegetable farm (Angelic Organics) in Caledonia, IL and also ran some comparative trials of different varieties. John Pounder, who farms organically in the Delavan, WI area, was a constant source of advice and produced hybrid seed for us on the Turtle Creek farm, located near Delavan.

Several Amish farmers in Southern Wisconsin tested an open pollinated variety (Multiline) which we licensed to John Pounder for production in all years of the project. Some of those farmers in turn, sold the open pollinated seed to other Amish farmers in 2016 and 2017. They also grew the topcross hybrid NG10 x S7 which John produced on Turtle Creek farm in 2017. Those larger scale tests are not recorded in diagram 1. However, two Amish farmers in the Darlington, WI area, who would prefer to remain anonymous, did yield trials or inbred seed multiplication or both.

In 2016 and 2017 we had our first winter nurseries in Hawaii with biodynamic/organic grower Marie Mauger (Anahola, HI). These trials focused on developing nutritionally enhanced, cross incompatible, N efficient corn for the Global South/Tropics and on multiplying early inbreds that were identified by graduate student Zach Paige as being yield competitive and high in protein and carotene for the Northern Corn Belt.

The University of Puerto Rico provided services on their organic farm in Lajas for a breeding nursery for two seasons (2015, 2017).

All of the above mentioned sites were organic; however, Steve Mohr/Foundation Seed Organic, also paid for winter nurseries in Chile with a conventional nursery (Semameris, Buin, Chile) to accelerate the production of inbred seed. In 2016 we received permission from the Ceres Trust to use some of our funds to pay for the winter nursery in Puerto Rico as we did not otherwise have funding for it.

For the small plot work, farmers listed in Diagram 1 often provided help with tillage and fertilization, and a lot of good advice.

Pierre Meyer (Alltech, Inc.), consults with farmers in the Upper Midwest on rations for thousands of organic birds. He graciously volunteered to estimate the feed value of our high methionine corn using data on the nutritional value of the grain relative to normal corn in the context of different scenarios.

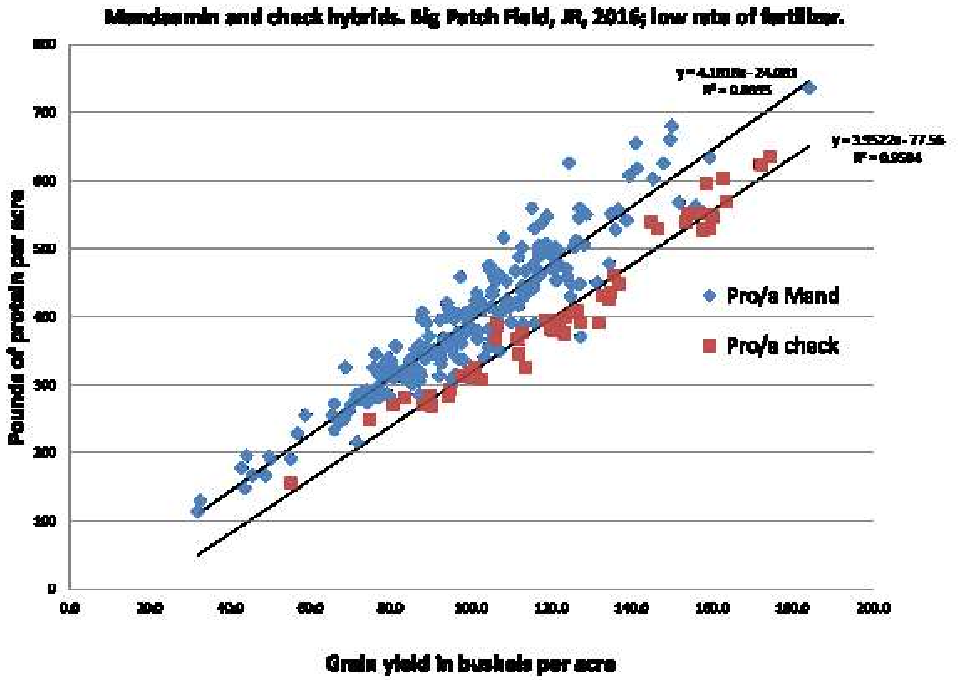
Steve Mohr from Foundation Organic Seed (Onalaska, WI) has given advice on developing the seed and has paid for winter nurseries in Chile to advance seed. He is also helping to market it.

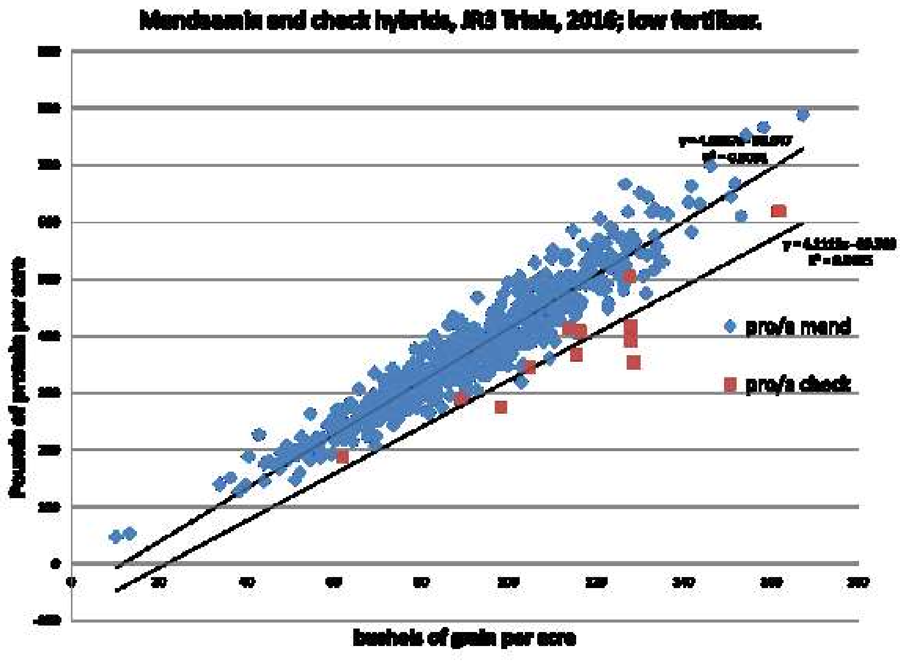
Research Results

Methionine, Protein, and Grain production. Yield trial results in 2015 and subsequent analysis of grain by USDA-ARS suggested that a number of Mandaamin hybrids produced competitive yields to hybrid checks (shown in red script) but had at the same time higher yields of protein, oil, methionine, and lysine both on a per acre basis and on a kernel basis (Appendix 1: Table 1 and 2). Crossing Mandaamin inbreds with inbreds from a commercial company produced high yields but often resulted in a decrease in quality.

Results from replicated trials on the Zinniker Farm (high fertility) and on the Rohrer farm (low fertility) showed that a number of Mandaamin hybrids had competitive yields and moisture contents at harvest to commercial organic hybrid checks in red script (Appendix 1, Tables 3, 4). The two experiments were not equivalent as there were less entries and replicates on the low fertility site. However, there are indications that some of those that yielded well on the low fertility site sometimes did not yield well on the high fertility site while some hybrids yielded well on both sites. Furthermore, Appendix 1, Table 5 shows results with N efficient hybrids brought back from the Chile nursery relative to commercial checks. These results are interesting because they suggest that several different kinds of N efficient hybrids may also yield competitively.

In 2016, hybrids were grown under very high and very low fertility conditions on two farms. Several of these hybrids yielded the same as hybrids from Blue River Hybrids and Prairie Hybrids seed companies (pp 17-23 in Goldstein, (2017)). In these trials some Mandaamin hybrids performed relatively well under both low N and high N conditions. But they produced up to 53% more protein/acre and more methionine and lysine in their grain than conventional hybrids under low fertilization conditions (Table 3). At the same yield level, Mandaamin hybrids produced more protein/acre than normal hybrids under N-limited conditions, demonstrating their enhanced N efficiency (Goldstein, (2017) pp 17-23). Under high fertility conditions, such as those met on the Zinniker farm with rates of fertilization with animal manure, there was relatively lower differences in protein/acre.





- **Graph 1 and 2. The two graphs above show the grain and protein yields of our hybrids (blue) compared with conventional hybrids (red) on two sites which received low amounts of fertilizer in 2016. There were 10-20% differences in protein yields at the same level of grain yield between our hybrids and conventional hybrids.**
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Table 1. Results in 2016 on two low fertilility and low fertilizer organic sites. Near Elkhorn, Wisconsin.					
Site and Pedigree	grain yield	protein	lysine	methionine	Protein yield
	bu/acre	--percent total dry matter--			pounds/acre
Rohrer, JR3 site, low fertilization rate					
Mandaamin ave 5 hybrids	125	9.00	0.36	0.26	535
Conventional ave 4 hybrids	115	6.97	0.30	0.19	381
diff NGSC vs checks in %	9	29	18	35	40
Rohrer, BP site, low fertilization rate					
Mandaamin ave 5 hybrids	150	8.88	0.36	0.27	627
Conventional ave 1 hybrid	124	6.96	0.29	0.19	411
diff NGSC vs check in %	21	28	20	38	53
Zinniker site very heavily manured					
Mandaamin ave 10 hybrids	178	11.28	0.39	0.30	951
Conventional ave 6 hybrids	180	10.40	0.33	0.25	885
diff NGSC vs checks in %	-1	8	19	23	7

N efficiency: Recently we have made observations that again suggest that this greater mineralization from soil organic matter is partly due to different root development. In 2017 we qualitatively inspected the rooting systems of the 12 top inbreds that we have developed versus conventional inbreds under N-limited conditions on two fields. There were differences associated with maturity as earlier corn had small root systems. But in general, conventional inbreds often had very tightly appressed, vertical roots (Photo montage 1). The concentration of secondary roots in the topsoil region was moderate to sparse. In contrast, the adventitious rooting systems of most of our inbreds are relatively spreading with broad crowns (Photo montage 1). This causes their most intensive zone of roots to encompass a larger volume of topsoil. Their adventitious roots are mostly densely covered with secondary roots in the top 6 inches of soil.

The rooting system on the conventional inbreds parallels their upright leaf habit. It may be the outcome of selecting under growing conditions with high population densities, where scavenging of fertilizer-N from water in the profile and resistance to root lodging are emphasized. The rooting systems of our inbreds is the result of selecting for plants that can utilize the topsoil to obtain N from soil organic matter. However, more systematic, quantitative research needs to be done to confirm whether these observations and hypotheses hold true on many sites.

Photo montage 1. Root systems in the top 8 inches of soil from conventional and Mandaamin inbreds grown under N limited conditions.

Conventional inbreds LH206, LH123, S7, S5 grown on JR or Creek field (S5) in 2017 without fertilizer. LH206 and 123 were bred by Monsanto; S7 and S5 are commercial inbreds from a seed licensing company.

Nokomis Gold Seed Company inbreds C4-6, LAT-7, NG2-3-2, and C2-B bred at the Mandaamin Institute and grown on JR or Creek field (C2-B) in 2017 without fertilizer.

By 2015 we had fixed what we believe to be the N_2 fixation trait into an inbred that had very dark green foliage when grown under N-limiting conditions on several sites (see Photo 1 and 2; Goldstein, (2016); (2017), pp 14-16).

Photo 1. 2015. Mex.6(2x)-1-B-4-sister lines 5,6,and 7, grown on the Goldstein Farm.

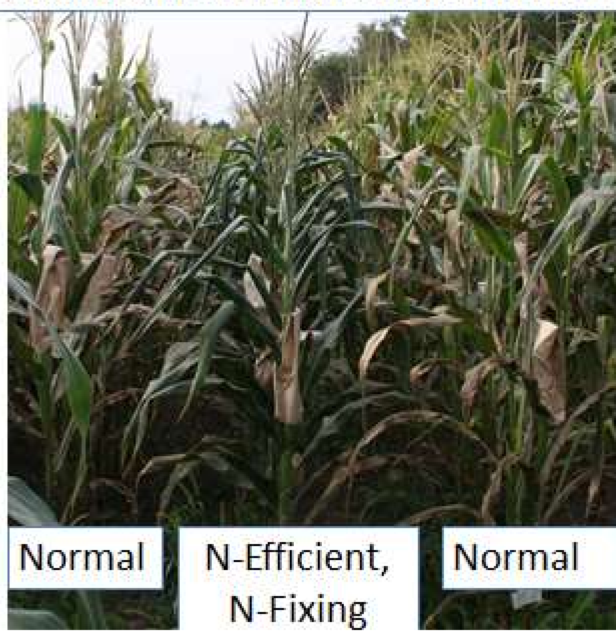

Photo 2. 2015. Corn on an unfertilized, Warsaw sandy loam in Little Prairie, Wisconsin after grass.

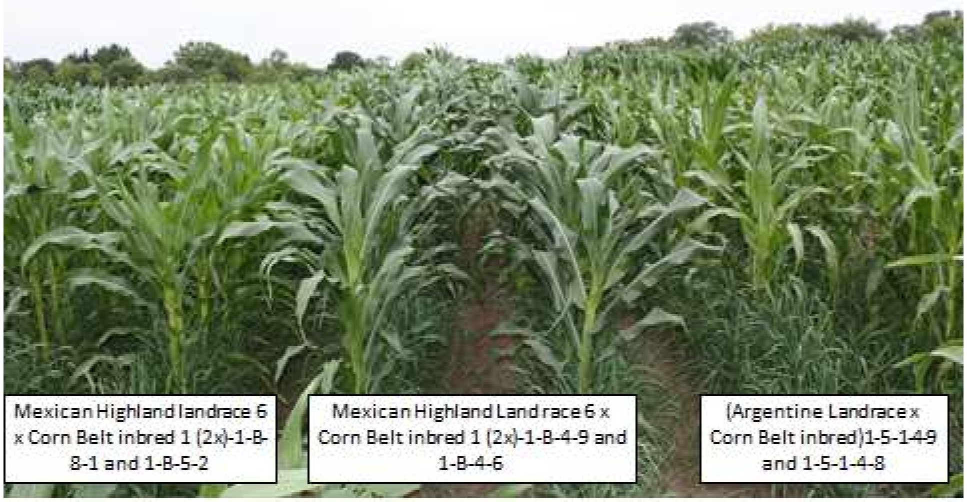

Photo 1 shows the inbred (which we now call C4-6 for short) next to sister lines that do not have the trait. Comparisons of advanced sister breeding lines that possess or do not possess the trait showed that the trait is associated with earlier flowering, and better ear set under drought and N stress conditions. Measurement showed that the 'N type' had darker foliage, faster growth rate before flowering, better synchronicity of flowering between anthesis and silking, fewer barren plants, but lower height than the sister lines that did not express the trait. Possession of the trait reflects profound physiological shifts that may result in a better adapted plant with a larger tissue N pool available for producing reliably high grain protein contents.

This C4-6 inbred was derived from what had been the most N efficient breeding family grown on a 1% organic matter soil in 2012. The breeding family had also shown a very high N content in its tissues in trials in 2014 on multiple sites and in subsequent years. High chlorophyll content in leaves is highly correlated with N content in the leaves. The high chlorophyll content which was apparent in all three years under soil conditions with limited N availability indicated to us that the corn might be N efficient or fixing N_2. Subsequent measurement of chlorophyll content has shown that these cultivars may have 15% to one third more chlorophyll than conventional inbreds. Efficient extraction of N from organic matter has been related in the scientific literature to the presence of large, extractive root systems. However, inspection of C4-6 indicated that the rooting system was relatively small, enough so that the plants were somewhat susceptible to root lodging.

Grow-outs of this inbred subfamily in 2016 and 2017 in winter and summer nurseries showed the same growth type and dark foliage coloration relative to other breeding lines when grown under N limited conditions (see Photo 3).

Photo 3. Fixation of the Nitrogen fixation trait manifests as more chlorophyll in leaves and more robust growth under nitrogen deficient conditions. This picture, taken in early July 2016, shows related inbred (S6) families grown on a nitrogen deficient field. The lines on the left appear to not be as highly nitrogen efficient as those on the right, but the two sets are closely related (Mex 1 x Inbred 1(BC) 1-B-6 family on left and 1-B-4 family on the right).

Immediately, we crossed this inbred with many other inbreds and breeding families. In 2016 we grew these hybrid crosses under conditions with low and high fertility, first in Puerto Rico in the winter, and then in Wisconsin in the summer. Some of the F2 generations made with this parent expressed dark foliage when grown under N limited conditions (Photo 3). Cursory observation was that some of the darkest F1 and F2 generations appeared when both parents were from our breeding program rather than when ours was crossed with conventional inbreds, though there were exceptions.

Selection Methods: Based on observation of root/microbial interactions we developed a method for identifying and selecting likely N2 fixing plants. Only specific breeds appeared to do the fixation, and these varieties appear on a whole plant basis to be the most N efficient (more chlorophyll, productive ears, protein and grain yield under N-limited conditions than conventional varieties). This selection method needs multi-year testing.

Grain Quality: Over time, opaque kernels began to appear in unexpectedly large amounts in families in which there was little or no opaque seed in previous generations. Studies with numerous families did not indicate that the trait segregated in any kind of clear Mendelian pattern. In some backgrounds it proved difficult to fix the trait. Opaque kernels also appeared in some commercial inbreds including LH123 and PHK42 without indications of outcrossing.

Between 2011 and 2016 we analyzed 1250 samples of our hybrids in comparison to 149 samples from hybrid controls grown in the context of 9 replicated trials on organic farms. On average our hybrids had grain with 0.274% methionine while conventional had 0.21%. This is a 30% difference. On average our hybrids had 10% protein while conventional had 8.6% protein for a 16% difference. The percent methionine in the protein ranged from 2.14% to 3.14% for the different treatments but regression showed that at the same protein level our grain had approximately 0.56% or 22% more methionine in its protein than did the conventional corn. This is critical for formulating protein efficient feed. Wet chemistry analyses confirmed the differences between our corn and conventional corn were of the same magnitude as those estimated by NIR (see Table 1). Gel electrophoresis confirmed that this quality change is due to shifts of protein composition, with a reduction in the content of alpha-zeins which have low contents of essential amino acids, to accumulation of beta and delta zeins with higher concentrations of methionine and cysteine, and non-zein proteins rich in lysine.

According to poultry nutritionist Pierre Meyer (Alltech, Inc.), our corn should save organic poultry farmers $32/ton of feed ($493 vs $525) by reducing the need for organic soybean meal and synthetic methionine. As margins for poultry production are tight this is a significant saving. According to its nutrient composition the corn should be worth up to $3.74/bushel more for organic poultry producers than normal organic corn when the latter is worth $9.83/bushel. This is a 38% increase in potential value.

Public Events

There was strong participation from organic farmers in each of our three field days held on participating farms with lunches. Attendance averaged about 40 each field day. There was a mix of different kinds of farmers, with significant representation from the Amish. Field days included a presentation and discussion session. We asked specific farmers (John Pounder, Mark Zinniker, Jim Rohrer) business advisors Tera Johnson (UW-Madison Food Systems), Jim Gage (James Gage Consulting), private and public breeders (Kevin Montgomery (Montgomery Consulting, Maroa, IL), Paul Scott (USDA-Ames, IA)), researcher Abdullah Jaradat (USDA-Morris, MN) to also present and attend. We wanted to get advice from the group on how to proceed in the development of our seed. At each field day the situation of the Mandaamin Institute was presented and farmers and business people gave valuable feedback. In addition, each December we had an open house to present our research results to a group of citizens which were mostly mixed consumers, business people, and farmers (see advertisement flier for 2017 event at the end of this presentation). Attendance was generally 25-40 people. These sessions became open discussion sessions where we were given advice on how to proceed in developing our project. Organic lunches were provided each year by the staff of Farmwise, Inc., on the Goldstein Farm. During these public events we also announced that the Ceres Trust had co-funded our work.

Presentation of our work and dialogue with colleagues and stakeholders occurred in various settings. These included significant oral presentations of our work at the:

- Seeds and Breeds conference, Washington DC, 2014;
- Organic Seed Alliance Conferences, Corvallis, OR, in 2014 and 2018;
- Biodynamic Association biennial conferences in Louisville, KY in 2014 and in Santa Fe, NM in 2016.
- Northern Plains Sustainable Agriculture Society meeting, Pierre, SD, in 2015;
- 19th International Symposium on Nitrogen Fixation, Asilomar, CA in 2015;
- Organic Agriculture Research Symposium at the MOSES conference, LaCrosse, WI, 2015;
- Indigenous Farming Conferences, Callaway, MN in 2016, 2018;
- Maa Viva Conference, Milwaukee, WI in 2016.

In conjunction with Mr. Woody White, a workshop on corn was given together with Ho Chunk people in Black River Falls in 2016.

Poster presentations were given at the Midwestern Poultry Congress, Minneapolis, 2016, and at the USDA-SARE conference in St. Louis, MO in 2018.

Furthermore, in conjunction with Dr. Abdullah Jaradat we produced our third peer reviewed scientific article in scientific journals on the quality of the grain from our breeding program.

Significance of Results:

Grain quality: In our program we have witnessed and are utilizing the spontaneous appearance of opaque kernels with high nutritional quality protein in breeding lines and inbreds. For our purposes these mutations are superior to the commonly used opaque 2 nor flowery 2. Why is this occurring? Possibly, conditions under which we are growing are inducing the plants to shift their genetic and epigenetic patterns. Low N-input organic farming may be conducive to the expression of higher quality protein, and conversely, selection for high yielding cultivars under high soluble N, conventional farming probably induces poor quality protein. We suspect that the opaque ‘emergence’ found in our program is due to general epigenetic shifts in zein production by the plants, induced by low N conditions (Mueller et al, 1997), shifting microbial relationships, by our selection processes, or some combination thereof.

There are other organizations with an interest in promoting a higher quality corn. Master’s Choice (Anna, IL) offers hybrids with an emphasis on softer kernels targeting dairy farmers. Our corn also has low density, floury kernels with high digestibility. CB Seeds (CB Seeds, 2017) emphasizes higher oil corn to boost the energy content of dairy feed. Thurston Genetics/BASF markets inbreds which produce Nutridense corn that is more nutritious for feeding pigs (Neutkens, 2006). However, Mandaamin hybrids have more methionine, lysine and cysteine and a higher % methionine in the protein than conventional or Nutridense hybrids (see Table 1).

Table 2 shows the value of Nutridense corn which is the only other high nutritional corn on the market relative to normal (conventional) corn (data from the web). We also show our wet chemistry data for Nokomis Gold corn relative to conventional corn. Our corn appears relatively higher in lysine, methionine, and % methionine in protein than the others.

Table 1 compares industry values for Nutridense and Nokomis Gold hybrids				
	conventional[1]	Nutridense[1]	conventional[2]	Nokomis Gold[2]
	percent total dry matter			
Protein	7.65	9.00	7.93	10.50
lysine	0.234	0.288	0.290	0.331
methionine	0.153	0.189	0.174	0.262
cysteine	0.171	0.198	0.174	0.215
%met in pro	2.00	2.10	2.15	2.46
[1] Data on Nutridense and conventional from Neutkens, 2006.				
[2] Data shows HPLC analysis of 3 conventional and 6 Nokomis Gold hybrids grown side by side in Wisconsin in 2011 (Goldstein, unpublished data).				

To our knowledge, no other company has the kind of grain quality that we have developed. Dairy farmers recognize that high levels of methionine and lysine and improved digestibility of corn in the rumen are equally important drivers of dairy production (Schwab, 2012; Seymour, 2016) and our corn possesses both traits. Use of our corn should reduce or eliminate the need for synthetic methionine supplementation widely used by dairy producers (1) and the organic poultry industry and enable their growth in the face of continued restriction of synthetic methionine (McEvoy, 2015; Fanatico and Ellis, 2016). An incentive is the enhanced value of the grain (up to $3.74/bushel more) for organic poultry producers than normal organic corn.

Nitrogen efficiency/fixation: The N efficiency of our hybrids is partly due to greater mineralization from soil organic matter caused by greater root length in the topsoil and to N_2 fixation. In small plot trials the best of our hybrids produced competitive yields with conventional checks in normally or heavily fertilized conditions ranging up to 200 bu/acre (19; Goldstein, 2017 unpublished data). But our limited data suggests they can produce up to 53% more protein/acre and more methionine and lysine in their grain than conventional hybrids under low fertilization conditions (Table 3). Based on N isotope and root research we believe their N efficiency is mostly due to them having both better root systems for extracting N from soil organic matter and to N_2 fixation. Utilizing our hybrids might reduce N fertilizer use. This will disrupt the N fertilizer-pollution cycle while increasing farm profitability, reducing environmental health hazards, and benefiting consumers

We do not know yet how use of our corn could affect the need for N fertilizers for conventional and organic farmers. However, N fertilizer use is a major global problem that clearly needs to be reduced.

There is a transgenic imperative to solve N efficiency/fixation**.** Apparently, there is little difference between Pioneer-Dupont hybrids in how they respond to N fertilizer (Luce and Mathesius, 2017; DeBruin and Butzen, 2017) so they are developing transgenic varieties. Obviously, a patentable trait that would help farmers reduce their fertilizer bill might quickly lead to market dominance. Thus, in the U.S.A. the major seed breeding conglomerates Pioneer Hi-Bred, Monsanto, and Syngenta are engaged in a costly race to develop 'super corn' that is highly N-efficient (Birger, 2011; Grooms, 2012). Dupont- Pioneer have a transgenic trait that conveys N efficiency and they predict they will have a product out within a decade (Luce and Mathesius, 2017; DeBruin and Butzen, 2017). Dow-Agroscience is pursuing a similar track together with Arcadia Biotechnologies (Arcadia, 2015). The Improved Maize for African Soils, (Gilbert, 2014), the Engineering Nitrogen Symbiosis for Africa (ENSA) project and Monsanto (Stephenson, 2015) are researching transgenic alternatives. Despite investments, based on internet searches we have not found that any genetically engineered N efficient corn hybrids are on the market or close to market. This research presupposes that the host cereal plant will eventually perform in an economically competitive way despite massive multi-genic transformations. Transgenic modification may result in imbalances leading to lower fitness and non-competitive yields (Shi et al., 2013; Gurian-Sherman, 2009) which may slow or prevent coming to market.

We published a bulletin in 2016 (Goldstein, 2016) on the internet on the Mandaamin website and on the website of Rural Advancement Foundation International (RAFI). The bulletin summarizes research done by the scientific community on N_2 fixation by corn and includes the results of research funded by the Ceres Trust and others. It is called: **Partnerships between maize and bacteria for nitrogen efficiency and nitrogen fixation**, Mandaamin Institute Bulletin 1. The intent of the bulletin is to inform the public and scientists about nitrogen fixing corn and how to breed for it. A second objective was to publish what has been done so as to prevent patenting of the natural N2 fixing trait. To our knowledge no patents have been granted yet.

Next Steps

We obviously have developed unique corn that needs to be taken to the next level. So far, we tested the outcomes on small scale plots under organic farm conditions. Breeding lines were screened on low N organic sites in small plots. Hybrids were produced and tested for yield on cooperating organic farms.

Our results have been discussed with scientists and both organic and conventional farmers. As the Mandaamin corn has evolved there has grown keen interest and numerous discussions within our team and with interested farmers about how to best move the project forward towards commercialization and widespread use. Mr. Pounder and I met several times with Amish farmers in South Central Wisconsin to discuss the project and those farmers have taken part in testing high methionine corn on their farms. As was mentioned above, Mandaamin hosted field days each year to showcase the research and to provide opportunity for interested farmers to discuss the project. Farmers viewed the N efficient cultivars, which are impressive because they are very dark green and vigorous relative to normal cultivars under N-limited conditions.

The consensus at the end of 2017 was as follows: First, that we need to do good strip testing and to document the impact of the N-efficient corn on fertilizer needs in the soil, on protein and methionine, on grain yields, and the feeding value of the grain and which hybrids have the best potential for commercialization. Mr. Mohr, Pounder, and myself have been moving hybrid seed production forward, enabling the high methionine corn to be tested on a larger scale with farmers in Wisconsin, Iowa, Illinois, and Indiana starting in 2018. Second: we need to accelerate development of the high methionine, N efficient corn complete with the cross incompatible trait. Though the high methionine and carotene, N efficient corn is ready for testing on selected farms, the combination that includes the cross incompatibility trait still needs more breeding and testing. This combination of traits is very important for organic dairy producers who are facing the need to reduce the level of GE contamination in their corn seed to be low (less than 0.2%) in order to meet the intensified demands of the marketplace. Third, we need to establish whether and under which conditions the corn/bactrerial association can fix N_2.

Outcomes: The corn plants in our breeding program have responded to our methods by throwing out a great deal of useful variation for grain quality and growing characteristics. We have done our best to select those plants that express those useful traits and are adapted to organic production. Outcomes are cultivars at different levels of inbreeding with combinations of all the traits, data on performance of cultivars, scientific papers on N fixation in corn and on corn nutritional value, and information presented on the Mandaamin website, annual field days, a publication, and outreach through organic farmer networks, and indigenous farming conferences. We found that some of our hybrids appear to produce competitive yields, better quality grain, and more protein per acre. These hybrids appear to be especially useful in conjunction with reduced N availability. This may make lower fertilization more attractive and reduce both farmer and environmental costs. We published a bulletin on the internet that summarizes the status of research on nitrogen fixing corn and that will help others to understand what N_2 fixation in corn is and how to breed for it. It will also help protect the public availability of the trait.

A set of farmers are testing our hybrids in 2018 alongside conventional hybrids. Eight hybrids are being tested. We are working with a seed company to produce and market seed. Two small scale egg producers who are our cooperators, are producing eggs without feeding synthetic methionine (Moses Beiler, Rewey, WI and Mark Zinniker, Elkhorn, WI). Results with seed production in 2018 are positive so far, but as planting occurred late we are hoping for a long, warm season. We and a nearby organic farmer (John Pounder) are producing seed for testing and production in 2019.

Further progress: In conjunction with a new OREI grant from USDA to the University of Illinois (Professors Michelle Wander, Martin Bohn), our high methionine, high carotene, N efficient cultivars are being tested on approximately 30 organic farms in Wisconsin, Iowa, Illinois, Indiana, and Colorado in 2018. As mentioned above, Steve Mohr from Foundation Organic Seed (Onalaska, WI) has sponsored two winter nurseries in Chile to help multiply seed and produce eight hybrids for that testing. That seed is being used in strip trial research. We will be having field days in Wisconsin and Illinois this year. Our hope is that these new hybrids will set a new standard for organic farmers.

In addition to this, a grant from USDA-SARE is enabling us to test the N uptake by our corn on several organic farms. Furthermore, a group of citizens including farmers, conservation agronomists, and citizens in Pepin County have started to test our hybrids on four farms in order to reduce N fertilizer use and thereby reduce nitrate pollution of well water.

We continue to refine our breeding lines and to test hybrids in order to determine what will be useful for farmers and seed companies and to provide corn with unique quality, N efficiency, and cross incompatibility to GE pollen. We need to find funding to continue that work next year or we may have to stop it prematurely.

Social and Financial Forms: Finding the right way to more the corn forward financially and socially is not easy. Our ethic and approach are different than other seed corn breeding companies. We are opposed to the use of our corn for making genetically engineered hybrids and opposed to patenting the naturally occurring traits in the corn. We have found that this does not make us attractive to some investors. Furthermore, funding for public breeding is very difficult to obtain, grant writing is time consuming and risky, and is uncertain as we are competing against large universities with greater prestige and political influence. Furthermore, we are concerned that our effort maintains a strong connection with organic farmers, and driven by their needs so that it does not suffer mission-drift. We have needed to look into the future and think about what kind of financial model might work for us, long-term.

Our first step was to form an LLC called Nokomis Gold Seed Company to license the corn to seed companies and promote its use.

Following multiple discussion sessions with farmers, Steve Mohr, and various other business people, we have also decided to try to create a Quality Crop Research Association based on the club approach taken by apple breeders at public universities. The purposes and functions of the Association include:

1) Eventually obtaining financial support from farmers and companies for the breeding work and research. This should be in the form of a) royalty payments from seed companies; b) farmers paying 10% more for seed; c) grain handlers/manufacturers paying 3% of gross grain purchases.

2) Sharing and protecting intellectual property in a responsible and equitable way: a) Only farmers and gardeners who are members can buy our seed, but any farmer or gardener can become a member. b) Joint ownership is based on exclusive use by members. c) Only companies that are members can buy the grain or produce/market seed. d) Members protect the IP by agreeing to not give away or sell seed as opposed to grain.

3) Providing a framework for marketing seed and grain.

4) Engendering farmer participation in research and development.

5) Helping other small scale breeders. Other breeders can join the Association for marketing their varieties and/or for partnering on breeding projects with shared royalty arrangements.

The overall intent of this Quality Crop Research Association is to

- Enable the success of small scale breeding ventures such as ours.
- Allow farmer and feed and food companies access to seed and grain with enhanced value for sustainable, healthy farming.
- Have members financially support, and have access to, quality oriented breeding programs
- Building a social and working framework that fosters the breeding, culture, and marketing of corn and other crops.
- Providing farmers the opportunity to participate in the development of quality grain as well its use and marketing.

The Association is owned initially by Nokomis Gold with help from Mandaamin, but may evolve to joint ownership with farmers and others.

Budget and Expenses. The funds obtained from the Ceres Trust were fully used for supporting the breeding project. Allocated funds and how they were used for the three years are reported in the Table below. The one significant deviation from the plan was use of the funds to support a winter nursery in Puerto Rico to increase seed for the second year of the project. That change was requested and approved by the Ceres Trust.

Use of funds from Ceres by the Mandaamin Institute

	year			
Category	**2015**	**2016**	**2017**	**Total**
Labor & Salaries	$ 47,694	$ 33,748	$ 44,800	$ 126,242
Travel	$ 2,970	$ 2,843	$ 2,373	$ 8,186
materials & PR nursery expenses		$ 15,362	$ 3,247	$ 18,609
Overhead	$ 7,046	$ 7,389	$ 9,682	$ 24,117
Total Expenses	$ 57,711	$ 59,342	$ 60,101	$ 177,154
Total funded	$ 57,724	$ 59,435	$ 59,995	$ 177,154
difference	$ 13	$ 93	$ (106)	$ -

Poster for Mandaamin Institute's open house, 2017, Lake Geneva, Wisconsin.

Art by Anne Decker, Hilbert, WI.

7TH OPEN HOUSE

AT MANDAAMIN INSTITUTE

WHEN: NEW YEAR'S EVE 12-31-17 AT 4-6PM

WHERE: MADAUS STREET #4, LAKE GENEVA, WI. 53147

GLADLY RSVP TO 262 642 9738

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Appendix 1.

Table 1. Summary of quality analyses for corn grown in three large experiments in Southern Wisconsin in 2015.

Top hybrid trial; JR1 field, Elkhorn, Wisconsin, 2015

Type of Hybrid	No. Samples	Protein Average	Protein std dev	Oil Average	Oil std dev	Lysine Average	Lysine std dev	Methionine Average	Methionine std dev
		% of total dry matter							
Mand x Mand	120	9.58	0.88	4.89	0.37	0.37	0.025	0.272	0.019
Mand x Conv	21	9.01	0.55	4.65	0.41	0.353	0.026	0.244	0.016
Conv x Conv	12	8.32	0.76	4.08	0.32	0.309	0.015	0.219	0.013
% diff Mand vs Conv		16.5		19.9		19.7		24.3	

N efficient hybrid trial; Zinniker field, Elkhorn, Wisconsin, 2015

Type of Hybrid	No. Entries	Protein Average	Protein std dev	Oil Average	Oil std dev	Lysine Average	Lysine std dev	Methionine Average	Methionine std dev
		% of total dry matter							
Mand x Mand	90	10.93	0.79	5.02	0.241	0.373	0.023	0.287	0.02
Conv x Conv	7	9.51	1.12	4.47	0.17	0.313	0.012	0.229	0.021
% diff Mand vs Conv		15		12.5		19.2		25.4	

New hybrid trial; Zinniker Farm, Elkhorn, Wisconsin, 2015.

Type of Hybrid	N. Entries	Protein Average	Protein std dev	Oil Average	Oil std dev	Lysine Average	Lysine std dev	Methionine Average	Methionine std dev
		% of total dry matter							
Mand x Mand	78	11.13	0.84	4.99	0.27	0.375	0.024	0.283	0.02
Mand x Conv	6	10.63	0.5	4.85	0.17	0.354	0.01	0.263	0.013
Conv x Conv	6	8.91	1.13	4.49	0.27	0.312	0.028	0.208	0.02
% diff Mand vs Conv		24.9		11.1		20.2		36.1	

Table 2. The nutritional composition and yield of nutrients at the Rohrer (JR1) farm in 2015. The hybrid check is shown in red.

JR1 2015. Summary of 2 row plot data. Top 21 hybrids												
Pedigree of Hybrid	**grain yld @ 15.5% moist**	**reps**	**Protein**	**Oil**	**Lysine**	**Met**	**Cys**	**Met + Cys**	**pro/acre**	**oil/acre**	**pro + oil/acre**
	bu/a		---------------------------%---------------------------						-----lbs/acre-----		
NG10-6-3-1/S7	199	1	9.3	4.6	0.34	0.25	0.19	0.44	778	386	1164
S7/8-4-1	192	3	8.8	4.9	0.36	0.24	0.19	0.42	704	394	1098
8-6/S7	191	3	8.7	4.6	0.35	0.24	0.19	0.43	673	357	1030
H-4/8-4-4	185	2	8.7	5.0	0.34	0.26	0.19	0.45	659	377	1036
8-4-1/S7	185	2	9.2	5.0	**0.38**	0.22	0.19	0.41	709	384	1093
8-4-1/NG10-6-1-B	181	3	9.0	4.6	0.35	0.24	0.19	0.42	680	344	1025
S7/8-6	173	3	8.9	4.4	0.35	0.24	0.19	0.43	633	315	949
Prairie Hybrids 3074	**172**	**29**	**8.2**	**4.0**	**0.31**	**0.22**	**0.17**	**0.39**	**611**	**296**	**907**
N.L.-17/8-4-3	170	2	10.6	5.1	**0.41**	**0.30**	**0.20**	**0.50**	722	347	1070
NG9-45-2-5/B2-22-2-9	170	1	9.2	5.1	0.35	**0.28**	**0.20**	**0.48**	626	351	977
6-B7/FS.NL	169	1	9.6	4.8	0.36	**0.28**	**0.20**	**0.48**	665	332	997
NG9-32-7-2/B-6-B-tall 6	168	2	10.4	5.2	**0.38**	**0.28**	**0.21**	**0.49**	679	341	1020
M-5-4/FS.NL-2	167	1	9.5	5.7	**0.37**	**0.28**	**0.20**	**0.48**	678	409	1087
8-4-2/NG10-3-1-1	166	1	10.2	4.9	0.36	**0.28**	**0.21**	**0.49**	698	335	1033
NG9-51-4-3/B-6-3-4	153	3	10.6	4.6	0.36	**0.28**	**0.20**	**0.48**	636	274	910
FS.NG/B-6-B-tall 5	152	3	10.8	4.8	0.35	**0.27**	0.21	**0.48**	673	299	972
M-5-B/H-2	149	3	10.5	5.4	0.35	**0.27**	**0.21**	**0.48**	645	330	975
M-8-6/NG10-6-1-B	148	2	10.7	5.3	**0.37**	**0.28**	**0.21**	**0.50**	641	320	961
NG9-35-5-B/6-B5	147	1	10.4	4.7	**0.40**	**0.28**	**0.21**	**0.49**	635	286	921
M-5-4/FS-14-8-3	135	2	10.6	4.9	**0.37**	**0.27**	**0.21**	**0.47**	626	288	914

Table 3. The top yielding 30 hybrids in replicated trials on the high yielding Zinniker Farm site, near Elkhorn, Wisconsin, in 2016. Inbreds shown in blue script are putative N efficient/N fixing inbreds.

no plots	Pedigree	bu/acre	moisture %	yld/moist
2	2.4)-4-1-5-5x **C-2-B-2-4-1**	197	22.2	8.9
4	**check 5200**	**192**	**20.6**	**9.4**
4	**check 5879**	**187**	**20.0**	**9.4**
2	2.4)-4-1-5-Bx **C-4-6-40**	186	20.0	9.3
2	C-2-B-2-5 x **C-4-3-45**	185	22.4	8.3
3	**check TR3622 x TR5435**	**180**	**18.9**	**9.5**
2	S7 x **C-4-6-10**	179	20.8	8.6
2	M-8-6 x **C-4-9-11**	176	22.7	7.8
2	NL-17 x **C-4-6)-1**	175	23.1	7.6
2	H-4 x **C-4-9-20**	174	22.0	7.9
2	C-6-B x **C-4-6-1**	173	**19.3**	9.0
4	**check 3074**	**171**	**19.9**	**8.6**
2	B-6-B-2 x **C-4-9-1**	170	20.8	8.2
4	**check GP373 x GP374**	**170**	**19.5**	**8.7**
2	C-6-2-B x NG10-11-B	170	20.3	8.4
2	**C-2-B-2-4** x **C-4-9-14**	168	25.1	6.7
	C-2-B-2-4 x **C-4-4-33**	166	24.0	6.9
2	5PLH)-1-7 x **C-4-9-13**	166	21.2	7.8
2	FNL-14 x **C-4-6-4**	164	20.9	7.9
2	9.2)-7-1-2-1-B x **C-4-3-34**	163	23.6	6.9
2	9.2)53-4 x **C-4-6-28**	161	22.9	7.0
2	2.4)-4-1-7-7x **C-2-B-2-4-2**	161	25.0	6.4
20	**check 3415**	**160**	**18.6**	**8.6**
2	NG10-11 x L-116-B-2-4-B	159	20.1	7.9
2	B2-22-2-4 x C-B-4-6-12	159	22.9	6.9
2	K42-20-4-9 x C-4-3-43	158	18.3	8.6
2	FNL)-12 x C-4-9-15	158	23.5	6.7
2	B2-3-2-1-1-2 x C-4-6-13	158	24.5	6.5
4	S5 x NG10	158	19.9	7.9

Table 4. The top 30 highest yielding hybrids on the Rohrer farm under low fertility conditions. This trial was carried out under conditions where little seed was available of different hybrids. Therefore, the conventional hybrid check Prairie Hybrids 3074 was replicated 48 times or every 8th plot. The other hybrids were replicated 1 to 2 times. Putative N efficient, N fixing inbreds in the pedigree are shown in blue.

pedigree	bu/a	moisture	yld/moist.
Cu-10 x C-4-3-32)	160	21.9	7.3
9.2)-11-2-2-4-1 x C-4-3-32	160	22.5	7.1
S7 x C-4-6-10	152	23.6	6.4
B-22-2-4 x C-4-6-12	150	25.2	6.0
K4N)-1-1-2-3-1-2 x C-4-3-42	145	23.6	6.2
9.2)-11-2-2-4-1 x C-4-14-2	141	24.6	5.7
FG-3-7 x C-4-6-22	141	22.7	6.2
B-6-B-4 x C-4-14-6	140	27.8	5.0
5PLH)-1-2)-1-8 x C-4-9-12	139	24.1	5.8
9.2)-15-2-7-3-3 x C-B-4-9-30	137	23.9	5.7
5PLH)-1-2)-1-7 x C-4-9-13	136	23.6	5.8
H-9 x C-4-3-32	134	21.7	6.2
2.4)-4-1-5-5 x C-4-6-40	131	23.0	5.7
PHN73.NG28)-51 x C-4-6-6	127	25.4	5.0
Cu7069-(2x)AR2-4)-2 x C-4-9-20	127	23.1	5.5
8-6-6 x C-4-6-10	126	19.8	6.4
9.2)-3-1-1-3-4 x C-4-14-30	126	22.4	5.6
C-2-B-2-4 x C-4-9-14	124	27.6	4.5
9.2)-11-2-2-4-1-1 x C-4-9-22	124	23.3	5.3
PH3074	**124**	**23.2**	**5.3**
B-6-B-1 x C-4-14-5	122	26.5	4.6
9.2)-8-2-1-4-1 x C-4-3-34	122	22.1	5.5
BS11 HMxBS31 HM)-2-1 x C-4-14-10	121	23.9	5.1
B-6-B-1 x C-4-14-6	121	30.0	4.0
C-6-3 x C-4-6-1	121	25.1	4.8
9.2)-7-1-2-1 x C-4-14-35	120	26.0	4.6
B-6-1-3 x C-4-14-3	119	26.5	4.5
L116-B-2-4 x C-4-9-10	118	27.3	4.3
9.2)-8-2-1-4-1 x C-4-14-32	117	25.7	4.6
B-6-B-15 x C-4-14-4	117	25.9	4.5
B-22-2-4 x C-4-4-1	117	23.4	5.0

Table 5. Yields of the top highest yielding 30 hybrids in replicated trials carried out on the Rohrer Farm in 2016 mainly with hybrids brought back from Chile that had one putative N efficient inbred parent (in blue script). Commercial hybrid checks are shown in red script. Entries were replicated three times.

Pedigree	Bu/acre	moisture %	yld/moist
T7-4 x FS-8-4-2	139	22.9	6.1
NG-2-3-2 x FS-8-4-2	133	21.7	6.1
NG-2-3-2 x FEMGEM-1-2-2	127	21.3	6.0
B-7-2-3-3-5 x FS-8-4-2	127	24.5	5.2
T7-4 x B-7-2-3-3-5	125	24.5	5.1
LT7-2 x C-4-6	123	21.2	5.8
NG10-11 x FNL-2-5	121	19.1	6.3
M-8-6 x FNL-2-5	121	21.7	5.6
PH3074	120	18.3	6.5
NG-2-3-2 x Md10AR-1-B-4	119	19.5	6.1
NG10-11 x FS-8-4-2	119	20.1	5.9
5PLH-1-8 x 8-4-3-B-2	119	21.1	5.6
PH3415	118	18.1	6.5
T7-4 x AR3-15-3-B-2	118	26.2	4.5
LT7-2 x FS-8-4-2	117	23.9	4.9
9.2)-15-2-7-3-2 x NG-2-3-2	116	20.9	5.6
LT-8 x L-116-B-2-4	115	21.7	5.3
APD)-S4-1-3 x C-B-4-6	114	20.0	5.7
LT7-2 x C-5-6	113	22.3	5.1
LT7-1 x C-4-6	113	21.2	5.3
LT7-8 x H-2-2	112	19.0	5.9
HAT-B x FNL-2-5	112	22.4	5.0
LT7-2 x 5PLH-1-8	112	20.3	5.5
NG10-11 x 8-6-4	111	18.3	6.1
H-2-2 x B-6-1-3	111	21.2	5.2
GP373xGP374	110	18.2	6.1
NG9 x P86-1-1-1-1	110	20.9	5.3
5PLH-1-8 x 8-6-4	110	17.8	6.2
PH5879	110	19.2	5.7
B-7-2-3-3-5 x 5PLH)-1-2)-1-5	110	20.0	5.5
LT7\1) x FS-8-4-2	109	24.1	4.5
P.47(2X)-4-7-B-1-B x C-4-6	109	18.5	5.9