

Special Section:

Fostering Socially and Ecologically Resilient Food and Farm Systems Through Research Networks

The evolution of a partnership-based breeding program for organic corn

Walter A. Goldstein* Mandaamin Institute SPECIAL SECTION SPONSORED BY:



U.S. DEPARTMENT OF AGRICULTURE

Submitted February 28, 2023 / Revised May 26 and September 1, 2023 / Accepted September 5, 2023 / Published online November 17, 2023

Citation: Goldstein, W. A. (2023). The evolution of a partnership-based breeding program for organic corn. *Journal of Agriculture, Food Systems, and Community Development*, *13*(1), 71–90. <u>https://doi.org/10.5304/jafscd.2023.131.011</u>

Copyright © 2023 by the Author. Published by the Lyson Center for Civic Agriculture and Food Systems. Open access under CC BY license.

Abstract

This paper describes decades of research developing a new type of corn (maize) cultivar that utilizes partnerships with seed-borne, bacterial endophytes to create environmentally friendly, nutritious corn that is better adapted to organic farming. Over

* Walter A. Goldstein, PhD, Executive Director, Mandaamin Institute; W2331 Kniep Road; Elkhorn, Wisconsin 53121 USA; +1-262-348-7534; wgoldstein@mandaamin.org

Conflict of Interest Disclosure

The salary of the author has been partially funded by competitive grants and has depended on successful conductance of various research and educational projects described in this paper.

Funding Disclosure

The various sources funding the research described in this report are reported in the text. In addition, the writing of the report was part of the outreach component supported by USDA-NIFA-OREI grant 13581782 called Corn/endophyte partnerships for organic farmers.

time the project engaged and formed multiple, evolving networks of corn breeders and other scientists, organic farmers, nongovernmental organizations (NGOs), private companies, the U.S. Department of Agriculture Agricultural Research Service (USDA-ARS), and state agricultural universities in several states. It addressed and partly resolved the need for developing (a) yield-competitive hybrids with greater nutrient density (methionine and minerals), (b) better adapted inbreds for organic production conditions, and (c) reduced pollution from nitrogen fertilizers.

The partnership approach taken also differs from usual top-down mechanistic breeding approaches in that the methods of breeding entailed holistic attention, learning, and respect for what turned out to be corn plants evolving in symbiogenesis with beneficial microbial partners. Initial studies indicate that the resulting corn from the program is competitive in yield but has better

Development of the JAFSCD special section in which this article appears, "Fostering Socially and Ecologically Resilient Food and Farm Systems Through Research Networks," was sponsored by INFAS and eOrganic and supported in part by the U.S. Department of Agriculture, National Institute of Food and Agriculture, through the Organic Agriculture Research and Education Initiative, Grant # 2017-51300-27115.

nutritional value. It obtains more of its nitrogen from microbial biomass and organic matter and nitrogen fixation than does conventional corn. Its performance partially depends on seed-borne plant/microbial partnerships. This corn continues to be developed at the Mandaamin Institute but is also being commercially introduced for testing by farmers.

Keywords

corn, maize, methionine, symbiogenesis, organic corn breeding, rhizophagy, nutrient density, nitrogen fixation, collaborative research

Introduction

Corn is the most produced cereal in the world. In the U.S., the Corn Belt Dent corn was developed from native southern dent and northern flint varieties. Its productivity was enhanced by the breeding and use of hybrids. Because of its inherent productivity, corn has come to assume a predominant role in US agriculture and in the production of feed and fuel. Crop rotations in the Eastern third of the country are rich in corn and this has been possible due to the use of synthetic fertilizers and pesticides. Corn is also the most widely grown organic row crop in the U.S. (USDA National Agricultural Statistics Service [USDA NASS], 2022), as well as a key ingredient for supporting organic animal production. The domestic organic corn supply is generally inadequate (Alonzo, 2016; Kowalski, 2017; McBride & Greene, 2015), with the deficit in supply met by imports of grain, sometimes with fraudulent consequences (Ross, 2018).

The corn breeds used today by breeding companies are largely derived from corn bred by public-sector breeders from state universities and from USDA ARS in the last century. These scientists were responsible for many of the innovations in plant breeding that have led to diverse and productive hybrids. The consolidation of seed companies by a few seed industry multinationals has led to greater investments becoming available for research and development. Considering industry's investment, and believing in the potential of genomic approaches, the USDA and universities phased out positions and funding sources for fieldbased public corn breeding while focusing funding on molecular analysis and technologies. Public and private breeders retired and took with them the culture of practical field-based corn breeding. Corn breeding became owned by industry and increasingly genomics and laboratory driven. As consolidation limits competition and farmer choice (Davies, 2022), there have been negative effects on farmers and society (Hacket, 2021). Since the turn of the millennium, the cost of corn seed has been increasing, and pollution from pollen drift intensified as the percentage of GM corn grown increased to over 90% (Center for Food Safety, 2023).

Today, the corn hybrids used by most organic farmers are bred by large private companies to perform under conventional conditions, in highdensity planting with high levels of synthetic nitrogen (N) fertilizers and pesticide applications (Mastrodomenico et al., 2018). However, this status quo is not a sustainable one for the planet. Dependence on N fertilizers means continued pollution of ground and surface waters with nitrate and continued high emissions of nitrous oxide, a potent greenhouse gas. Furthermore, although conventional hybrids are high yielding and readily available, they provide only a partially good fit for the needs of organic agriculture. Gains in grain yields are due to the industry investing in intensive breeding and testing programs that develop plants that produce high yields wherever they are planted, including under stressful conditions. However, the parental inbreds developed in such programs when grown for hybrid production under organic conditions often lack vigor, tending to be nutrient deficient and to compete poorly against weeds (Goldstein et al, 2012). Furthermore, organic inbred, hybrid, and grain production all have problems with genetically engineered (GM) contamination, soil nutrient limitations on organic farms (especially N), climatic instability, and weeds. The hybrids available to organic farmers depend on nitrate and ammonium producing fertilizers. Organic farmers often use chicken manure as a fertilizer to address N needs of corn, but it is expensive, potentially polluting, and often derived from non-organic confinement operations.

Furthermore, modern corn hybrids have

increased yield potential but inadvertently have led to inadequate nutritional value; grain protein content decreased from 10.3% in 1940 to a projected 7.4% in 2010 (Duvick et al., 2010). Organic poultry farmers need to have corn varieties with a high content of the essential amino acid methionine in their protein, to enable the production of animals without depending on feeding them synthetic methionine.

New cultivars targeted to thrive under organic conditions are needed. This paper describes the evolution of a partnership breeding approach with corn that has been directed toward making up some of the deficiencies associated with conventional corn. Begun in 1988/1989, the program has survived and evolved only by participating in multiple networks of different kinds. Admittedly, the crucial central partnership is a newly evoked breeding relationship with the emergent, creative biology of corn and its symbiotic endophytic microbes. But other key partnerships involve other organic corn breeders, farmers, scientists, NGOs, seed companies, universities, the USDA, and various private and public funders. The partnership has depended on informal networks that have been active on different scales and at different times for different ends. Those networks have shifted in accordance with the maturity and needs of the program and the interests of farmers and others, as will be described.

The paper falls naturally into four parts. The early phase describes the inception of the program and how it evolved into cooperative breeding and testing networks, as well as opportunities that emerged with the Seeds and Breeds Movement. The second part describes initial efforts to improve nutritional quality and nitrogen efficiency, two traits desired by organic farmers, while maintaining adequate grain yields. The third part describes the results from multiple research partnerships that deepened our capacities to improve corn as well as our understanding of how to work with corn to improve its quality and environmental friendliness. Finally, we reflect on the potential value for society of what has been achieved, on barriers to further corn development, and the need for even broader partnerships to enable the full potential of organic corn.

Part 1. The Program's Inception, Including its Early Efforts, Philosophy, Partners, and Funding

I began my employment as research director at Michael Fields Agricultural Institute (MFAI) in the fall of 1986 after finishing my doctorate in agronomy at Washington State University. My entry into corn breeding started with a field day on Dave Christenson's organic farm in Jefferson County, Wisconsin, in 1988, where I was surrounded by a group of seven organic-friendly farmers who requested that I begin to breed open-pollinated corn for them. Their experience was that openpollinated corn had better nutritional value-more minerals and protein, and better taste. The better quality was confirmed by their experiences with animal preference by horses and cattle. However, the open-pollinated corn they had grown did not stand well in the field at modern planting densities; they suggested that I could remedy that.

Subsequently, I visited the late Albert A. Arens (1904-1988) at Green Acres Seed Company in Hartington, Nebraska. Arens bred and sold corn. He was renowned in certain organic farming circles for selecting plants that performed well without any fertilizers, and he also had a specific program devoted to breeding corn that produced multiple ears on each stalk. This resonated for organic farmers, as they also produced corn without synthetic fertilizers and some of them tended to produce corn under conditions in which the multi-ear trait would be useful. At the time of the visit with Arens in 1988, he was terminally ill with cancer and was concluding his last year growing corn. He showed me his breeding families and discussed corn breeding. At the end of the visit, he announced, "You are pregnant with an idea; you are going to become a corn breeder!"

I was challenged by these experiences and recognized the valid need for corn with better nutritional value. Despite there being no obvious longterm funding sources, I decided to try to grow different kinds of corn and develop open-pollinated corn with improved nutritional value and better standing ability. In 1989 I contacted several experienced corn breeders: Dr. Arnel Hallauer of Iowa State University, H. Z. Cross of North Dakota State University, and James Coors of the University of Wisconsin-Madison, and obtained seed stocks from them. I also received seed posthumously from Arens' prolific work from his nephew, and from Mark Millard at the USDA ARS Plant Introduction system (Mark Millard). Other significant contributors included a gift of corn seed from several Hopi farmers. Altogether, those stocks included both native corn varieties and Corn Belt dent (field corn with substantial soft starch content) varieties and populations.

From the beginning of the project, an attitude that emphasized partnership has played an important role in its success (Goldstein et al., 2019). I have not pursued the sort of mechanistic, reductive, top-down approach to produce uniform products as quickly as possible, which is common in the breeding world. Rather, I regard the maize plant as a biologically creative partner in dialogue with the breeder as in a kind of slow, multiyear dance. In this conceptualization, the proper human role is to establish useful conditions for evolution and selection, pay diligent attention to the evolving bodies of the plants as they grow in the field, make insightful observations, collect relevant data, and make selections that favor both plants and humans as the corn plants continue to evolve. The human role involves thinking, progressive learning, and gradually developing informed and accurate perception and judgment. This approach involves evolution of both the plant and the breeder. It brings into the breeding process the perspective of an I-Thou kind of relationship to supplement the usual I-It relationship, the latter defining plants simply as objects to be used (Buber, 2023). The I-Thou attitude is comparable to that of the Native people who were the original stewards of corn (Kimmerer, 2013).

Starting in 1989, for 14 years I carried out a small part-time selection and breeding program in the MFAI test plot fields and on my own farm, to provide seed for farmers who wanted to keep their own seed. I made crosses and developed new open-pollinated corn with improved yields, enhanced quality, and good standing ability. The program served an informal network of approximately 20 farmers who wanted to keep their own seed stocks. Participants mainly came from Michigan, Wisconsin, Iowa, Indiana, Missouri, and Ohio, with a few from several other states. The farmers obtained seed by just paying for the shipping charge, under the agreement that they would give the program feedback on their results.

The effort often entailed educating farmers how to grow, select, harvest, and maintain the corn populations. Instruction occurred mostly on a oneto-one basis over the telephone or at field days in Wisconsin organized by MFAI and in Iowa organized by Practical Farmers of Iowa (PFI) to include on-farm selection. Of the farmers, Don Adams from Madrid, Iowa, and Dan Specht from MacGregor, Iowa, took the most active interest in breeding, selecting, and feeding the corn. One of the most significant populations that came out of working with them was Nokomis Gold. Nokomis was developed from a mixture of Southwest native corns from the Hopi, Zuni, and Navajo tribes and included some of Arens' corn; it played an important role in further development of the corn breeding program. Dan Specht continued his own breeding work, and by the time of his untimely death in 2013 he had selected a polenta population from Nokomis Gold and other seed stocks provided by the project.

Partnership with USDA ARS

Around 2000, I was involved in joint research with Kendall Lamkey of the USDA ARS in Ames, Iowa, and Zeno Wicks III of South Dakota State University. We were testing different open-pollinated varieties with a grant from the USDA Sustainable Agriculture Research and Education (SARE) program. I was also taking part in educational events at PFI dealing with corn quality and relationships between corn, soil, and nitrogen. In 2001, at a field day on the Adams farm sponsored jointly with PFI and USDA ARS, seed of Nokomis Gold was made available to farmers in larger quantities. A winter field day was also held at a Northeastern Iowa State University research station. Dr. Lamkey showed results from the University breeding program, and I showed results from the Michael Fields Agricultural Institute trials based mainly in Wisconsin. Cornbread was baked using flour from several different corn varieties and hybrids and offered in a blind taste test. The variety Nokomis Gold was the preferred source of corn. However, the yield of

corn from the Michael Fields corn program still lagged considerably behind that of hybrid corn.

In 1999 I was advised by the MFAI administration to stop breeding the open-pollinated corn because there was no sustaining source of funding for it. In response. I requested assistance from Senator Herb Kohl of Wisconsin and from Senator Tom Harkin of Iowa. Senator Harkin was approached with assistance from Robert Karp, then executive director of PFI. With the help of the senators, it proved possible to obtain an appropriation in 2003 that allowed MFAI to continue research on corn together with USDA ARS and PFI at Ames, Iowa.

Cooperative Breeding and Testing with Organic Farmers

The time around 2000 was tumultuous for many organic corn farmers because there were problems with contamination from the pollen of genetically engineered hybrids onto organic corn, which could negatively affect the sales of organic grain and organic seed. As it seemed very important to ask farmers which direction the corn breeding research should take, in 2003 the I organized an open session at the Upper Midwest Organic Farming Conference (now known as the Marbleseed Organic Farming Conference), one at the Minnesota Organic Farmers Conference, and one at the annual PFI conference. The objective was to discuss what kind of corn these organic farmers wanted, not just what they did not want. The sessions were well attended, with about 40 to 70 farmers at each event.

The results were consistent across meetings. While the farmers clearly did not want genetically engineered corn contamination, in addition they wanted corn that did well under their systems, which often had less available nutrients, especially nitrogen. They wanted corn that competed well with weeds. They also wanted nutritional quality, especially those farmers who fed their own livestock. Only a few farmers wanted open-pollinated varieties from which they could keep their own seed. Most of them wanted hybrids, because the open-pollinated varieties did not yield enough. The overall principle for them was that the corn they used should have better quality, but it should also produce at least 90% of the yields attained by conventional hybrids.

The experience of the MFAI breeding and testing program to that point had been that competitive yield levels could only be obtained by utilizing hybrid vigor. I then decided to focus most of my effort on breeding for hybrid production, while continuing some, but less, work on open-pollinated populations. Soon afterwards, in 2003, Linda Pollack from USDA ARS took over the USDA breeding program from Dr. Lamkey as he moved into the ISU breeder position. Dr. Pollak had helped found and direct the USDA Latin American Maize Project and the Germplasm Enhancement of Maize Project, which were both concerned with utilizing the potential of exotic corn landraces (Pollak, 2003), so she possessed experience breeding populations into commercial grade inbreds. I obtained additional counseling on the procedures needed for breeding inbreds and producing hybrids from Dr. Kevin Montgomery, who had decades of experience developing hybrids at three large commercial companies and subsequently advised the MFAI project.

The USDA ARS-Pollak program worked on both breeding for organic farming and improving grain inherent quality. In conjunction with the program, the MFAI staff attempted a holistic, cooperative, farmer-engaged, variety-development and seed-production model. An immediate question was what kind of hybrids should be bred. Producing hybrid corn by crossing inbreds is difficult for farmers to do for themselves because the inbreds are short, lack vigor, and demand special care. However, making hybrids by crossing vigorous populations was feasible for farmers and offered them the financially attractive possibility of easily producing their own hybrid seed. The USDA and ISU programs had experience with producing varietal hybrids that produced competitive yields under organic conditions, even if such hybrids lacked superior nutritional value. Therefore, the MFAI and USDA programs jointly engaged in breeding new, vigorous, genetically narrow populations and testing them in combination with other vigorous, narrow populations (varietal hybrids) or with inbreds (top crosses).

An example of the cooperation was the devel-

opment of a significant breeding family. The Nokomis Gold variety developed by the MFAI program was grown for many years on the Adams farm and was selected by Adams and me using stratified mass selection. John Golden, a technician from the Ames, Iowa, USDA ARS breeding program, also made selections from it that showed good combining ability in hybrid trials. These selections were subsequently further bred in Wisconsin to produce LMPNG28 breeding lines, which showed good per se productivity and combining ability in hybrids. Inbreds from the NG10 branch of that family are still in use in Mandaamin Institute's breeding, testing, and commercialization program.

Testing and farmer field days took place on several organic farms in Wisconsin: Mark and Randy Hoffman's farm in Whitewater, WI, Nokomis Farm in East Troy, WI, the Zinniker farm in Elkhorn, WI, Skip Kaufman and Jessie Niggerman's farm near Eau Claire, WI, and the MFAI farm in Troy Center, WI. In Iowa, test plots from both programs were grown on three to five organic or low-input sites each year. Outreach, field day, annual event, and organizational support was provided by PFI. A detailed account of the farmers involved in the MFAI portion of this partnership, the fields and practices used, selection and breeding methods, breeding philosophy, and the targeted trials and outcomes for yields, agronomic traits, and quality is available (Goldstein et al., 2019). The pedigree breeding program, which is mainly used to produce inbreds, entails sequential inbreeding and selection for plant performance and grain quality on N-limited farm sites, coupled with early testing of hybrid combinations on multiple sites.

Although several varietal or top-cross hybrid combinations seemed to have promising yield performance, there were two problems. First, they had somewhat higher grain moisture contents at harvest. Second, organic seed companies expressed disinterest in them because they were not as uniform as single cross hybrids made by crossing clone-like inbreds. Due to the second issue, over time both programs moved in the direction of single cross hybrids with good combining ability. It became increasingly clear, however, that it might be important to select the inbreds for performance under organic conditions. A substantiating observation made by both breeding programs was that many inbreds raised conventionally did poorly when grown under organic conditions. They appeared to lack vigor and were nitrogen deficient. However, when grown repeatedly under organic conditions, some appeared to adapt and improve both vigor and nitrogen uptake, a phenomenon I later studied and confirmed (Goldstein et al., 2019). It was attributed to genetic and epigenetic shifts and to shifts in the populations or the activity of endophytic microbes.

The Seeds and Breeds Movement

Parallel to these developments was the emergence of a movement fostering awareness of the impact of seed industry consolidation, restrictive patents on seed available to farmers, and the general decline in publicly sponsored breeding and seed diversity. Michael Sligh from the Rural Advancement Foundation International-USA, working with an organizing committee composed of concerned public breeders, including Professors Kendall Lamkey (ISU), Bill Tracy (University of Wisconsin), Charlie Brummer (University of Georgia), and myself, organized several conferences on these themes in Washington, DC, and at Iowa State University. Efforts were made to interact with USDA competitive grant authorities to open the door to funding public breeding activities. Initially, it appeared to be an uphill battle to create an avenue beyond genomic research that would enhance seed availability and public breeding. Eventually, the work of the political arm of the sustainable agriculture movement led to important changes, most significantly an emphasis on breeding in the new USDA competitive grant organic agriculture funding program. This made it possible to maintain my corn program and many other worthwhile public breeding efforts.

Part 2. Improving Quality, N Efficiency, and Microbial Partnerships

The Search for Better Grain Nutritional Quality In order to diversify and find better sources for breeding, MFAI also began a partnership with the USDA Germplasm Enhancement of Maize (GEM) project. The GEM project aimed at developing useful diversity by crossing with exotic corns possessing attributes not present in Corn Belt corn. The GEM team initially was primarily concerned with improving protein quality. In addition, Paul Scott of the USDA ARS unit in Ames was also actively breeding for improvement of protein quality.

The need for improving the quality of corn was explored at MFAI in different ways. A great deal of research by the international community of corn scientists had been devoted to improving protein quality, with special focus on the lysine content in grain. The international corn breeding center in Mexico, CIMMYT, had devoted much effort to using the opaque-2 gene for that purpose. Early experiments we did in Wisconsin testing opaque-2 in crosses confirmed that it was not well suited for commercial production because of its effects on vields, the fragility of kernels, and that it was a recessive factor that could easily be deactivated by contaminating pollination. The more pressing concern of the organic community was the amino acid methionine, which is a component of protein (Fanatico & Ellis, 2016). Methionine is the most seriously deficient amino acid in poultry feed. Because the organic poultry industry is restricted in its use of synthetic methionine, the organic community has needed to find methionine in other feedstuffs, as the USDA may phase out the use of synthetic methionine (McEvoy, 2015). Corn with a high methionine content could substantially help organic poultry farmers. Therefore, the project focused initial efforts on testing the *floury-2* gene because it increased the methionine content of grain considerably.

We pursued three lines of research (Goldstein et al., 2008). One was the introgression of the *floury-2* gene into several open-pollinated populations with good combining ability. The second was testing a set of high methionine breeding lines identified through cooperative work with the GEM program. The third was developing a rapid nondestructive test for methionine in corn so that we could breed for the trait.

The task of developing high methionine corn entailed discussions and cooperation with the

Methionine Task Force, a consortium of up to 15 large organic poultry companies. We worked with Dr. Charles Hurburgh and his colleagues at the Iowa State University Grain Quality Lab to develop a near infrared spectroscopic calibration that would allow us to rapidly detect methionine, lysine, and cysteine (Goldstein et al., 2019; Hardy et al., 2009; Jaradat & Goldstein, 2013). The calibration broke the inherent correlative relationship between protein and amino acids (proteins are composed of amino acids) which had previously prevented effective calibrations. This was only possible because we used a calibration set of samples that possessed protein with different percentages of methionine, lysine, and cysteine in their protein, so results did not depend exclusively on the level of protein in the grain.

Corn varieties selected simply for high levels of protein generally increased α-zein storage proteins, which have lower concentrations of the essential amino acids (Darrigues et al., 2006; Frey, 1951; Tsai et al., 1992; Wu & Messing, 2012). Feeding such protein to poultry can be expected to lower feed efficiency and increase ammonia emissions in poultry litter (Burley et al., 2016). The high methionine varieties received from the GEM program were high protein, hard-kernelled corns (Goldstein et al., 2008). Higher levels of methionine content in the grain were due to a high protein content; so the overall quantity of methionine in the grain depended on achieving a high protein content in the grain. Trials with farmers showed that to sustain the methionine content from farm to farm with these varieties because the protein content of the grain varied from site to site. In addition, we were informed by industry and by Dr. Mark Cook at the Poultry Science Department of the University of Wisconsin-Madison that high protein content in corn was not desirable due to problems with ammonia emissions from manure. What was needed was a high percentage of methionine in protein and a moderate level of protein.

The soft-kernelled, *floury-2* corn had a considerably higher percentage of methionine in its protein. It could produce high percentages of methionine in protein when the protein content of the grain was low. This provided greater stability in methionine amounts across farms. Feeding trials

took place with our *floury-2* corn hybrids. The first set of trials with broilers was done by Organic Valley Coop (Levendoski & Goldstein, 2006). A second set of trials, funded by Organic Valley Coop at the University of Minnesota, involved feeding up a layer flock and validating one cycle of their egg production (Goldstein et al, 2012; Jacob et al., 2008). In both cases the high methionine corn produced the same amount of poultry product as was achieved by feeding normal organic corn plus synthetic methionine.

Unfortunately, our *floury-2* varieties were too low-yielding for commercial use. Homozygous *floury-2* caused approximately 11% lower weight per seed. This reduction conditioned a corresponding reduction in grain yields. Even without *floury-2*, the yields of our cultivars were insufficient; there seemed to be no hope for farmer acceptance in the future with an additional 11% yield drag.

A third path emerged spontaneously in our breeding families with the unexpected occurrence of soft kernels in anomalous amounts (Goldstein et al., 2019). These mutations became apparent in 2006 and continued to emerge in multiple varieties. They were found in Nokomis Gold, in multiple GEM breeding lines (Jaradat & Goldstein, 2013; 2014; 2018), and in ex-plant variety protection inbreds. Their soft kernels were associated with reduced contents of the poor quality storage protein α -zein. But they had enhanced overall levels of better quality storage proteins that resulted in both higher percentages of methionine and lysine in grain and also in protein (Goldstein et al., 2019). In most cases, the soft, floury trait did not seem to be associated with a reduction in grain size or yield. They possessed moderate levels of protein (approximately 10% of the total dry weight) but compensated when protein was lower by producing a higher percentage of methionine in the protein.

These soft kernelled mutations have continued to emerge during the breeding program (Goldstein et al., 2019). Whenever they did, they were channeled into a path for inbred development that included sequential self-pollination, and selection for opaque kernels. Plants were grown and selected mainly under unfertilized conditions, with low quantities of nitrate and ammonium, to help encourage plants to find ways to utilize microbial sources for nitrogen.

We hypothesized that the plant regulatory systems would shift in response to our breeding and selection practices, and that some of those shifts would be epigenetically inherited (Goldstein et al., 2019). Subsequently, the trait may become stabilized by major gene action in a Mendelian fashion. Mutable inbreds in the three major heterotic patterns (LH123 in the Lancaster pattern, PHK42 in the Iodent pattern, A632 in the B14, stiff stalk pattern) all showed the same path of development in our program. This path involved: (a) spontaneous development of seeds showing opaque or piebald (opaque and translucent) seed; (b) occasional occurrence of chimaeras and other phenomena associated with transposon (jumping gene) activity in the foliage or grain; and (c) instability of the trait according to growing conditions. In the case of some of the unstable lines developed from these three inbreds, opaque floury kernels were expressed by plants when they were grown under N-limited conditions, but translucent seed were produced under conditions where corn was heavily fertilized with mineral N. In other cases, the trait was initially stable, or repeated selection led to stabilization of the soft kernel trait. In some lines, tests for allelicity showed that the trait is identical to the *floury-1* allele. This was a surprise, because previous research by others (Holding, et al., 2007) had not shown that floury-1 causes higher methionine content in grain, but only a rearrangement of storage proteins in the protein bodies in the endosperm. In addition, we found that segregation ratios in crosses with nonfloury corn were highly irregular and inconsistent. On the other hand, the accumulation of the methionine-rich δ - and β -zein storage proteins in the grain depends on the supply of sulfurcontaining methionine from the plant. All this suggests that regulatory systems, a structural gene, and causal increases in the supply of methionine available to growing seed from the plant are jointly involved in the transition to production of the opaque phenotype in the grain of our cultivars.

The impact of the opaque kernels on nutritional value of the grain was studied with Abdullah Jaradat, USDA ARS, of Morris, MN, and professor at the University of Minnesota-Morris. The soft kernel trait was also associated with higher density of macro- and microelements in the grain (Jaradat & Goldstein, 2018), especially, C, N, K, Mg, Mn, and P (Jaradat & Goldstein, 2013). Breeding lines and inbreds from the breeding program were screened for an accumulation of mineral nutrients in 2019 alongside a panel of commercial inbreds, and similar results were obtained as before, although the differences were most positive for the Mandaamin lines for K, Cu, Mg, Mn, and Zn (Goldstein & Jaradat, unpublished results, 2022). Later research confirmed that the link between opacity and mineral content is somewhat variable and not fully nutrient specific but seems to have to do with a general increase in nutrient availability to the developing seed (Goldstein et al., 2019).

The Search for N-Efficient/N-Fixing Corn

Separate nitrogen budgeting studies were carried out by the MFAI team for conventional hybrid corn grown on organic farms. The studies showed that in some cases there was excess N uptake that did not parallel mineral nitrogen and organic matter turnover rates as were currently conceived (Goldstein & Cambardella, 2008). These findings led to questioning whether variable interactions with N-fixing organisms might explain these discrepancies. Furthermore, our on-farm trials had shown that the protein content of grain was unstable from site to site. Perhaps inoculation with nitrogen-fixing bacteria might help stabilize protein content. In 2008, inoculation of MFAI cultivars with two Azospirillum species provided by the TerraMax company of Minneapolis increased grain protein content by approximately 1% under unfertilized conditions, but not under fertilized conditions (Goldstein et al., 2019).

In 2009, I grew cultivars with *Azospirillum* inoculum but under N-limiting conditions on an organically managed field that had been in cereal crops and had not been fertilized for at least four years (Goldstein et al. 2019). Cultivars tested included: (1) 15 ex-commercial inbreds or S1 to S3 generations of crosses between them, (2) 23 new commercial advanced breeding lines/inbreds from a cooperating seed company at the S5 to S7 level of inbreeding, (3) 26 breeding lines from the MFAI organic breeding program (S1 to S3), and (4) 13 exotic landraces obtained from the USDA ARS Plant Introduction Research and selected because of their unusually high methionine and lysine content. Groups 1 and 2 had been previously selected under conventional management. Group 3 was in development mainly from populations grown for multiple years under organic management.

The chlorophyll content of leaves parallels N content to a high degree, so determination of chlorophyll content with meters is generally used as a quick, practical way to determine whether the N uptake by the corn plant is sufficient or deficient. Leaf chlorophyll scores averaged 37 for the older commercial inbreds or recent breeding lines derived by crossing them, 40 for the new commercial breeding lines which were close to being finished inbreds, 45 for the early MFAI breeding lines derived from organically managed populations, and 54 for the exotic landraces. The percentage of lines within each group with scores of 50 or over (showing N sufficiency) were 8% for the conventional lines/inbreds, 34% for the MFAI breeding lines, and 65% for the exotic landraces. Multiple races from Mexico and South America produced dark green leaves with levels of chlorophyll scores in the high 50s and low 60s, suggesting that they had been heavily fertilized. Several of these varieties had invested less in root dry matter than adjacent conventional lines, so the difference could not easily be explained by having larger, more extractive roots.

Grains were tested for isotope composition utilizing the natural abundance method (Boddey et al., 1991). The decreased $\delta^{15}N$ signatures on N from grain samples indicated that some of the dark leafed landrace cultivars might have fixed up to half of their nitrogen from the air (Goldstein et al., 2019). On the other hand, isotope results also suggest longer-term selection of breeding lines under biodynamic/organic conditions increased in $\delta^{15}N$ isotope ratio in the grain and tops, which indicated greater accumulation of N from microbial biomass (Craine et al., 2015). Nitrogen fixation in corn has been shown occur by others (see review by Goldstein, 2016; and recent research on a Mexican landrace done by Deynze et al. 2019).

Understanding Microbial Partnerships and Rhizophagy

Following publication of results in 2019, I was contacted by Dr. James White, a plant biologist at Rutgers University, New Brunswick, NJ, who thought that he could explain many of my results with the concept of rhizophagy. Rhizophagy is a recently discovered, and not well understood, widespread plant/bacterial partnership associated with nutrient acquisition by plants (White et al., 2018). Endophytic bacteria live and multiply by budding in the periplasmic space of root cells located in root tips (White et al., 2018). The bacterial cells are buffeted by reactive oxidative substances (ROS) secreted by host cell plasma membrane oxidases. As the root cells age, the bacteria lose their cell walls and become naked protoplasts. Oxidation of the bacteria degrades cell walls and bacterial membranes, releasing proteins and minerals that are absorbed by the host cell. The surviving bacteria in older root cells stimulate production of root hairs. They are subsequently expelled by cyclosis out of root hair tips into the rhizosphere, where they grow cell walls again. Rhizophagy strongly stimulates root hair production, root branching, the production of root tips within which bacteria multiply, and N2 fixation as measured by 15N gas uptake by seedlings (White et al., 2018, 2019a, 2019b; unpublished research). The bacterial species involved in rhizophagy originate from the seed itself, but fresh soil bacteria are recruited into root cells behind the root tip in the zone of maximal root exudation. ROS induced by this partnership stimulates higher levels of plant resistance to stresses (Choudhury et al., 2017; Kandel et al., 2017). ROS in turn induces microbial production of methionine as a protective mechanism against oxidation (Arts et al., 2015; Luo & Levine, 2009), and thereby might affect methionine accumulation by plants. The increased supply of methionine, and hence its accumulation in non-zein and β - and δ zeins instead of α -zein proteins, may explain the opaque phenotype and why it disappears in some opaque lines when the plants are fertilized with mineral sources of nitrogen. Hybrids that respond to N fertilizer have been found to respond to mineral N fertilization by increasing the concentrations of α - and γ -zeins and translucence in their kernels (Tsai et al., 1992).

Disinfection of the seed-borne bacteria associated with rhizophagy strongly reduced root branching and uptake of macro- and micronutrients (Irizarry & White, 2018; Verma, Kingsley, Bergen et al., 2017, Verma, Kingsley, Irizarry et al., 2017; Verma & White, 2018). Disinfection also stopped N₂ fixation in seedlings of maize (White et al., 2015; White, unpublished research).

The White lab at Rutgers found a high incidence of rhizophagy and enhanced root hair production in the roots of Mandaamin seedlings from different inbreds that were axenically grown. The putative N₂-fixing inbred C4-6 stood out among Mandaamin inbreds in appearing to have aggregates of bacteria embedded in a biofilm. Conventionally bred inbreds that were grown in the same breeding nursery lacked any sign of rhizophagy (Goldstein et al., 2020). These results supported earlier unpublished work by Dr. White.

Part 3. Research Coalitions and Their Achievements

Organic Corn Breeding Coalitions and the Formation of the Mandaamin Institute

During the first decade of the millennium, I made efforts to work with other small-scale corn breeders in a cooperative way. The efforts included seed exchanges with Frank Kutka, who bred early openpollinated populations for northern climates in Wisconsin and North Dakota, often using exotic corn in his crosses, and with Carl Barnes from Turpin, OK, who selected native corn for distribution back to Indigenous people. The most robust relationship was with Herman Warren, a retired professor of plant pathology, internationally known for his work in breeding disease-resistant corn, who had worked for USDA ARS at Purdue University and at Virginia Tech University. Dr. Warren bred for decades, adapting Mayorbela, an extremely disease- and insect-resistant landrace from Puerto Rico to cultivation in the Midwest and Southeast. I was encouraged by his results: despite generations of inbreeding, many of Dr. Warren's diseaseresistant inbreds maintained substantial vigor and still displayed some useful variation. I developed a

cooperative crossing and breeding program with Dr. Warren and also utilized the inherent instability in his lines for selecting opaque kernels and adapting for shorter-season Wisconsin conditions. Following Dr. Warren's death in 2015, I continued to work with his family to continue the cooperative breeding project at the Mandaamin Institute and to develop commercial grade inbreds and hybrids.

In 2009/2010 a coalition was formed with public breeders Linda Pollak (USDA ARS), Margaret Smith (Cornell University), and Richard Pratt (Ohio State University), and with private breeder Kevin Montgomery to seek funding together for a cooperative research program from the new Organic Agriculture Research and Extension Initiative (OREI) Organic Research funding opportunity, with Dr. Pollak as the principal director for the proposal. The grant was obtained, but in 2008 other USDA funds were reallocated away from the portion of the Pollak program that was devoted to corn quality research, leading to insufficient funds to support her program and necessitating some kind of consolidation. In 2010 Linda Pollak retired. Her role was taken over by Dr. Paul Scott, a USDA crop geneticist, who also managed a more lab-oriented program concerned with selecting populations.

In 2011 the breeding program under my direction was transferred to the Mandaamin Institute in Elkhorn, WI, which was founded by myself, in order to take the work further. The appropriation funding stayed with MFAI and USDA. The Mandaamin Institute was fortunate at this time to be receiving continued financial support from the NIFA grant, the Ceres Trust, private supporters, and local organic farmers. The support of the Ceres Trust has been especially critical for ensuring the continuation of the project to the present. The coalition of breeders continued their breeding and testing work together on all sites until 2020. The first grant received by the team (USDA NIFA OREI 2010-02363), "Strengthening public corn breeding to ensure that organic farmers have access to elite cultivars," ran 2010-2014. The main accomplishment was building the infrastructure required to support a public corn-breeding effort for organic production systems. Crucial infrastructure items included:

- The United States Testing Network (USTN), a cooperative testing network that carries out agronomic evaluations of corn hybrids in both conventional and organic environments. This network gave public breeders and small private breeders access to evaluation resources comparable to those of large plant-breeding companies. This network became self-supporting.
- 2. A certified organic winter nursery, established with researchers at the University of Puerto Rico near Lajas, allowed corn breeders to produce two generations of corn a year, doubling the rate of gain of the programs that used it. An eOrganic webinar (Brunner et al., 2014) documented our approach to carrying out this winter nursery, which served as a critical resource for our second award.
- **3.** A joint catalog of germplasm available to public researchers, which was the foundation for the breeding efforts carried out in subsequent breeding programs. Other products of the proposal included breeding families in various stages of development, 16 peer-reviewed scientific publications and other publications, and two graduate students and several undergraduates trained. Results were disseminated in field days, a website, and in lectures by the PIs.

The second grant received by the team (USDA NIFA OREI 2014-05340), "Breeding noncommodity corn for organic production systems," ran 2014–2019. This project took advantage of the infrastructure developed in the previous proposal to develop a germplasm pipeline for organic producers.

The focus of our breeding efforts was on traits of value to organic producers, including nutritional quality, native insect and disease resistance, N efficiency, ability to exclude GM pollen, and specialty varieties for food and feed. The cornerstone product of this project was a breeding study in which the best inbreds from each program were combined to form interinstitutional hybrids, which were evaluated by all cooperators (Huffman et al., 2017). This study informed decisions about how best to use the germplasm resources of the group in cooperative breeding efforts targeting the specific traits of interest. Major products of the grant included inbreds at various stages of release that combine high methionine and cross-incompatibility traits needed by organic farmers, two germplasm releases, release and farmer use of three highmethionine, open-pollinated varieties and several high-methionine hybrids in larger-scale strip trials and production by farmers and one organic seed company, 14 peer-reviewed scientific publications, seven other publications, and a webinar on corn breeding. Two graduate students and many undergraduate interns received training. Results were disseminated through field days, our website,¹ webinars, and lectures and meetings featuring the principal investigators (PIs).

Another important accomplishment was elucidation of the molecular mechanism of the pollen exclusion trait by the Scott lab (Lu et al., 2020). Contamination by pollen from GM plants is a serious threat to organic seed producers, farmers, and consumer perception that organic feed and food is non-GM. One option for reducing risk is using the genes Ga1 and Tcb1, found in teosinte (wild corn), in landraces of sweet and field corn, and in popcorn from Central America (De la Cruz Larios et al., 2008; Padilla Garcia et al., 2012), which convey gametophytic incompatibility or cross incompatibility. They minimize the chance of pollination by pollen that does not possess the same alleles. Corn can avoid GM contamination if it has these genes in a homozygous or, more rarely, in a heterozygous condition. Therefore, an area of common interest for three members of the team was developing commercial-grade varieties that had the crossincompatibility trait. In particular, Paul Scott attempted to develop a genetic marker system for the cross-incompatibility genes. Cornell University, the Mandaamin Institute, and USDA ARS also developed cross-incompatible breeding lines. Our program was substantially assisted in its efforts to develop cross-incompatible corn through cooperation with Dr. Major Goodman of North Carolina

State University, who had transferred a combination of *Tcb1* and *Ga1* from Mexican sweetcorn to Corn Belt corn (Jones, 2018) and helped us to transfer it from there to our corn.

Team members presented results from the project both in academic papers (Goldstein et al., 2019) and at academic and organic farmer conferences. Margaret Smith presented results at a NY Certified Organic Conference in 2020 and at an organic field day in 2019. In 2019, I presented results to farmers at organic conferences in Springfield and Champaign, IL, and in Shakopee, MN. All sessions included feedback from farmers. In 2019 findings of the Mandaamin research on breeding with microbes were presented at the Crop Science Society of America meetings, and Richard Pratt presented on landrace corn at the Rocky Mountain Seed Alliance Grain School.

Cooperating farmers participated in on-farm trials in MN, NM, NY, ND, SD, WI; in annual summer field days and winter open-house events in MN, NY, ND, WI, and in seasonal grower and university site visits in NM. WI events were attended by 40 to 90 people, mainly farmers. They included presentations from multiple OREI team members (Montgomery, Scott, Goldstein) followed by 1-2-hour discussion sessions to obtain feedback. In 2020, I presented information on the project to Northern Plains Sustainable Agriculture Society (NPSAS) farmers. Trials were run on five organic sites in MN, ND, and SD with earlymaturing hybrids from the Mandaamin program in conjunction with NPSAS farmers and North Dakota State University. Members of the NPSAS Farmer Breeding Club and the NPSAS board attended field events and met in a special retreat with me.

Through field days and my talks, farmers have articulated a keen interest in obtaining seed and seeing our programs evolve to meet their needs. A few farmers in WI who have been testing the high methionine corn have reported that they want cultivars that combine competitive yields, high methionine and carotenoid content, N efficiency, and cross incompatibility to pollen from GM plants. GM contamination of corn can cause milk proces-

¹ <u>http://eorganic.info/cornbreeding</u>

sors to drop farms or shipped grain to be rejected, and restrictions have been tightened. Two organic farmers—Mark Zinniker, Elkhorn, WI, and Moses Beiler, Rewey, WI—are growing the highmethionine corn and have fed it, without synthetic methionine, to small flocks of layers. Beiler (personal communication, 2019) reported that egg production went up in comparison to feeding with a balanced commercial organic diet that included commercial hybrid corn grain plus synthetic methionine inputs.

Interaction has occurred with large and small companies, including those engaged in organic seed, feed, poultry production, and grain marketing. Long-term dialogue has occurred with a consortium of organic poultry companies, the Methionine Task Force, about the need for highmethionine corn hybrids that can yield competitively with commercial hybrids. Feedback from several large feed producers and consumers indicates that many large organic poultry companies seem afraid to use the high-methionine corn, probably because if they do the USDA will ban the use of synthetic methionine.

Extension and NRCS

The Mandaamin Institute has been testing its Nefficient/putative N₂-fixing corn with organic and conventional farmers, UW-Extension, NRCS personnel, and a citizen-based water quality group in Pepin County, WI, led by Dr. Micheal Travis. There is interest in reducing nitrate contamination of well water. Farmers have been encouraged by yield results with Mandaamin hybrids, when fertilizer inputs were reduced.

Seed Companies

During the period of the two grants and beyond, the Foundation Direct Seed Company (FDS) in Onalaska, WI, has helped the Mandaamin Institute to multiply seed of high-methionine, N-efficient inbreds and has produced hybrid seed in winter nurseries. In 2020, FDS and the Mandaamin Institute grew approximately 50 acres (20 hectares) of inbred or hybrid production seed corn for farmer trials near Onalaska and East Troy, WI. Since 2020, FDS has been marketing four of the Institute's high-methionine hybrids. The Mandaamin Institute is also allowing its inbreds to be used for breeding purposes by several European companies.

CASH Project

The Mandaamin Institute participated in a USDA NIFA OREI-funded grant managed by the University of Illinois (2017-02413), "Participatory breeding and testing The networks: A maize based case study for organic systems," that ran 2017-2022. Also called the Corn and Soil Health (CASH) project, the integrated multiregion project conducted advanced on-farm research to identify biophysical and social and legal factors influencing the performance of organic maize cultivars and their dependent businesses.² It was a participatory process that incorporated end users into research and Extension activities done in concert with eOrganic. The objectives were to strengthen the organic seed supply and develop client-oriented breeding networks and business structures that deliver highyielding, nutritious, N-use efficient, and weedcompetitive genotypes adapted to organic systems.

The CASH project built a participatory testing and breeding program, conducted a maize-based case study to explore on-farm factors influencing crop fitness and grain quality, and identified how closing the loophole on allowing organic producers to purchase conventionally grown hybrids might promote client-oriented breeding programs to improve organic seed supply. Important inputs included promising cultivars, experienced advisors, and significant farmer insights. On-farm research was carried out on corn hybrids and synthetics, while structured experiments were carried out to assess the effects of soil health and plant-soil interactions on crop fitness and grain quality.

In 2018 and 2019, the Mandaamin Institute hybrids were tested in IL, IN, and WI for yield and quality in strip trials on 32 sites. The high-methionine and carotenoid corn hybrid differed clearly from commercial hybrid checks by delivering significantly more methionine, carotenoids, and trace minerals at comparable yield levels. Methionine levels in the Mandaamin corn averaged 43% to

² https://cornsoilhealth.web.illinois.edu/wp/

57% higher than for the conventional corn. Dietary studies showed that use of the high-methionine corn in poultry diets would reduce the need for organic soybean meal and synthetic methionine and would enable a premium for grain that would displace some potential yield losses (University of Illinois, 2020; Goldstein et al, 2023). The value of extra carotenoids and minerals for poultry was not calculated.

The SARE Project

From 2017 to 2022, the Mandaamin Institute also carried out a project funded by USDA NIFA SARE, "Testing N efficient, high methionine corn hybrids with organic farmers," which utilized strip trials in tandem with the OREI:2017-02413 grant to do additional research on relationships between Mandaamin hybrids and nitrogen and mineral uptake dynamics. Results have been published in the form of a SARE report (Goldstein, 2022). The hybrids were studied in the context of different farming systems and soil fertility conditions. Studies with microscopy, field trials on different farms, and mineral and natural isotope analyses produced five key findings: (1) the plants exercised rhizophagy cycles with seed associated bacteria, leading to N-efficiency in field trials, high levels of δ^{15} N in tissues, and grain with higher protein and especially higher mineral contents; (2) these partnerships result in yields comparable to manured commercial hybrids, but no manure is added to the Mandaamin hybrids; (3) Hybrids with the Mandaamin inbred C4-6 as a parent particularly express these traits and also appear to fix N2; (4) The C4-6-based Mandaamin hybrids also respond negatively to fresh manure (from various animals) but positively to high soil organic N and to high soil protein levels resulting from cattle manure applications in preceding years; (5) The negative effect of fresh manure applications on the C4-6-based hybrids extended to yield and mineral uptake, a problem exacerbated on soils with low organic matter content.

The work was made possible with the help of many organic and biodynamic farmers. USDA ARS at Morris, MN (A. A. Jaradat, Chris Wente, and Jane Johnson) did tissue analysis of corn for minerals and helped prepare samples for isotope analysis. Foundation Direct Seed Company (S. Mohr) contributed seed and advice. Rutgers University (J. White, A. Lotfi, K. Kingsley, and others) contributed rhizophagy research on maize seedlings, advice on interpreting our results, and continuous inspiration based on their research findings. University of Wisconsin Extension (Mike Travis) and Pepin County Conservation (Chase Cummings) helped organize farmer events and meetings around the issue of N₂ fixing corn and helped carry out on-farm research in NW Wisconsin. Wood Ends Soil Testing Lab and Cornell University assisted with extra soil quality tests. The Ceres Trust helped fund the project.

For some time, it remained a mystery why changes in the texture of grain should be associated with higher contents of nutrients, including minerals. Our results parallel findings at Rutgers that plants obtain extra N and minerals from microbial biomass in their roots (rhizophagy) while fixing N in their foliar tissues (Chang et al., 2021; Micci et al., 2022; Chang et al., 2023). Endophytes in foliar tissues and plant hairs are also bathed in oxidative substances, and nitrate is released around these bacteria. This dialogue of plants and microbes could explain both nitrogen fixation and the accumulation of methionine in grain, as both nitric oxide and methionine are potent antioxidants that can foster bacterial survival in an oxidative environment. We hypothesize that methionine production by the bacteria makes more of it available to the plant and increases the supply available for storage in grain proteins (Wu & Messing, 2012).

A New Project

A new OREI-funded grant, "Partnership breeding of corn for organic production systems," is enabling research from 2022 to 2024 together with Rutgers University and others. Recent research at the White lab at Rutgers has outlined the potential biochemical interaction between plants and endophytes living in epidermal trichomes and in epidermal cells and that this generates incorporation of nitrogen from the air (Chang et al., 2021; Micci et al., 2022). Initial research efforts show that within growing Mandaamin inbreds, nitrate-secreting bacteria are living in vascular tissues, in the profuse hairs in our cultivars, and in epidermal cells in leaves and husks. The convoluted epidermal cells in the cultivars can harbor large colonies of bacteria that stain for nitrate production. In some cases, the bacteria in these cells have also been found to be bathed in ROS as in root cells. The bacterial consortium responsible for the trait has been transferred to conventional corn that does not have these bacteria. We do not yet know whether transference of this microbiota will carry over to the next generation of plants.

A new organically managed winter nursery has been established in Chile. Recent inspection in Chile of the Mandaamin inbreds bred for N efficiency suggest that the effects of evolution and selection are causing a convergent development of phenotypes in breeding families from widely different genetic backgrounds. Seedlings tend to possess profuse, even bottlebrush-like root hairs, while mature plants have healthy, profusely branched rooting systems in the topsoil. As selection and adaptation progress, plants are appearing that tend to produce hairy to pilose leaves and leaf sheaths, and thick, often folded and buckled leaves with high chlorophyll contents.

Part 4. The Value of the Effort and the Findings

The research results we have shown, and especially the return to utilization of endophytes (Chang et al., 2023) has substantial implications that could help US corn production to become more sustainable.

Context and Significance

This paper has documented the progression of a program that has produced nutrient-dense corn that obtains its nitrogen from microbial sources. The survival and development of the program has depended on numerous temporary partnerships between dedicated people and institutions, with relatively low investments in research funding and professional commitment by the publicly funded agricultural system.

To understand the significance of the findings, we should consider corn in the context of the present world situation. To live more sustainably and to mitigate climate change, humanity needs to find alternatives to the massive use of energetically and monetarily expensive and nonrenewable nitrogen fertilizer. Its breakdown product, nitrate, pollutes our groundwater and rivers and lakes, creates hypoxic dead zones in the oceans, and releases the potent greenhouse gas nitrous oxide into the atmosphere. Our political-economic system is currently locked in a paradigm that causes this systematic pollution. Conventional thought leads to no practical solution or major regulation that can reverse the trend. Corn, both the most productive cereal and the primary consumer of N fertilizer, has been selected for a century to depend on nitrate fertilizers. Corn is also the major field crop for organic farmers who use inputs such as chicken manure to provide the nitrate and ammonium that corn needs to produce high yields.

The Mandaamin Institute has bred corn that seems to grow well with neither direct applications of manure nor nitrogen fertilizer inputs. The partnership approach differs from the usual top-down mechanistic approaches in that it involved holistic attention and respect for what has turned out to be corn plants evolving in symbiogenesis with microbial partners (Goldstein, 2022; Chang et al., 2023). Initial studies indicate the resulting corn is competitive in yield, has better nutritional value, and obtains more of its nitrogen from microbial biomass and organic matter than does conventional corn. Its performance depends on plant/microbial partnerships that are seed-borne. These results are significant for breeders of other crops, who could utilize the philosophy and methods to improve quality and reduce needs for fertilizer and pesticide inputs.

There are multiple barriers to overcome. Yields of the better hybrids lag 10–15% behind conventional hybrids under fertilized conditions, although yields for the Mandaamin hybrids may be better under unfertilized conditions. This yield drag can possibly be overcome by more effort to improve the heterotic potential of the Mandaamin breeding lines through breeding that improves the combining ability of inbreds, measured by yield-oriented selection and greater emphasis on yield trials. The higher nutritional value of the corn results in savings for the poultry farmer in feed costs, with reductions in need for soybean meal and synthetic methionine. Results from the CASH trials showed that the extra nutritional value in cost of feed could be transferred to the grain producer, enabling a sufficient markup in price of grain to offset the lower yield.

Another barrier is that some larger-scale organic poultry producers may be afraid that use of the Mandaamin corn will replace the need for synthetic methionine. Synthetic methionine is a relatively cheap input for farmers. Indeed, Mandaamin corn has the potential of reducing or eliminating the need for synthetic methionine. If the use of the high-methionine corn becomes widespread, as a consequence the USDA may then further restrict the use of synthetic methionine for feeding organic poultry. This would make organic poultry farmers dependent on high methionine corn, which might be more expensive, but would enable cost efficient feed due to a reduction in the need for organic soybean meal (Goldstein et al., 2023). Progress on this front depends on the proactive work by organic poultry industry and by USDA.

A third major barrier is the regulatory loophole

in organic regulations that allows organic farmers to purchase conventionally-grown, non-treated hybrid seed. This loophole needs to be closed as it results in reduced investments in organic seed production as farmers purchase cheaper conventional seed from companies that do not have a demonstrated interest in breeding corn for organic agriculture.

Despite its obvious potential, efforts to further improve and implement the use of such corn as we have developed have received minor support. They deserve even stronger partnerships and investment in order to reduce problems with N fertilizer overuse. Organic poultry farmers need highmethionine, high-carotenoid corn grain, while organic corn producers need high-yielding, highmethionine corn hybrids that yield competitively. Therefore, it is important that more significant players in the agricultural industry begin to support the testing and introduction of the new kind of corn in the farming world, to take it to the next step.

References

- Alonzo, A. (2016, March 1). Infographic: Feed shortage limits organic ponltry sector growth. WATTPoultry. <u>https://www.wattagnet.com/broilers-turkeys/article/15516728/organic-poultry-production-growth-hurt-by-feed-shortages-wattagnet</u>
- Arts, I. S., Gennaris, A., & Collet, J.-F. (2015). Reducing systems protecting the bacterial cell envelope from oxidative damage. *FEBS Letters*, *589*(14), 1559–1568. <u>https://doi.org/10.1016/j.febslet.2015.04.057</u>
- Boddey, R. M., Urquiaga, S., Reis, V., & Döbereiner, J. (1991). Biological nitrogen fixation associated with sugar cane. *Plant and Soil, 137*(1), 111–117. <u>https://doi.org/10.1007/BF02187441</u>
- Bruner, B., K. Montgomery, P. Scott. 2014. A Certified Organic Winter Nursery for Corn Breeding. eOrganic webinar Dec. 16, 2014. <u>https://eorganic.org/node/12866</u>
- Buber, M. (2023). I and Thou (Smith, R. G., Trans., Centennial ed.). Simon & Shuster.
- Burley, H. K., Anderson, K. E., Patterson, P. H., & Tillman, P. B. (2016). Formulation challenges of organic poultry diets with readily available ingredients and limited synthetic methionine. *Journal of Applied Poultry Research*, 25(3), 443– 454. <u>https://doi.org/10.3382/japr/pfw012</u>
- Center for Food Safety. 2023. About genetically engineered foods. <u>https://www.centerforfoodsafety.org/issues/311/ge-foods/about-ge-foods</u>.
- Chang, X., Kingsley, K. L., & White, J. F. (2021). Chemical interactions at the interface of plant root hair cells and intracellular bacteria. *Microorganisms*, 9(5), Article 1041. <u>https://doi.org/10.3390/microorganisms9051041</u>
- Chang, X., Young, B., Vaccaro, N., Strickland, R., Goldstein, W., Struwe, L., & White, J. F. (2023). Endophyte symbiosis: Evolutionary development, and impacts of plant agriculture. *Grass Research*, 3, Article 18. <u>https://doi.org/10.48130/GR-2023-0018</u>
- Choudhury, F. K., Rivero, R. M., Blumwald, E., & Mittler, R. (2017). Reactive oxygen species, abiotic stress and stress combination. *Plant Journal*, 90(5), 856–867. <u>https://doi.org/10.1111/tpj.13299</u>

- Craine, J. M, Brookshire, E. N., Cramer, M. D., & Hasselquist, N. J. (2015). Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. *Plant and Soil*, 396(1–2), 1–26. <u>https://doi.org/10.1007/s11104-015-2542-1</u>
- Davies, S. (2022, June 22). Seed industry debates consolidation, intellectual property protections. *Agri-Pulse*. https://www.agri-pulse.com/articles/17887-seed-industry-debates-consolidation-ip-protections.
- Darrigues, A., Lamkey, K. R., & Scott, M. P. (2006). Breeding for grain amino acid composition in maize. In K. R. Lamkey & M. Lee (Eds.), *Plant breeding: The Arnel R. Hallauer International Symposium* (pp. 335–344). Blackwell Publishing. <u>https://doi.org/10.1002/9780470752708.ch24</u>
- de la Cruz Larios, L., Sánchez-González, J. de J., Ron Parra, J., Santacruz Ruvalcaba, F., Baltazar Montes, B., Ruíz Corral, J. A., Morales, R., & Moísés, M. (2008). El factor gametofítico-1 (ga1) en híbridos comerciales de maíz de México [The gametophyte factor 1 (ga1) in Mexican commercial hybrids of maize]. Revista Fitotecnia Mexicana, 31(1), 57–65. https://www.redalyc.org/pdf/610/61031108.pdf
- Deynze, A. V., Zamora, P., Delaux, P.-M., Heitmann, C., Jayaraman, D., Rajasekar, S., Graham, D., Maeda, J., Gibson, D., Schwartz, K. D., Berry, A. M., Bhatnagar, S., Jospin, G., Darling, A., Jeannotte, R., Lopez, J., Weimer, B. C., Eisen, J. A., Shapiro, H.-Y., Ané, J.-M., & Bennett, A. B.(2018). Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. *PLoS Biology*, *16*(8), Article e2006352. https://doi.org/10.1371/journal.pbio.2006352
- Duvick, D. N., Smith, J. S., & Cooper, M. (2010). Long-term selection in a commercial hybrid maize breeding program. In J. Janick (Ed.), *Plant breeding reviews, vol. 24, part 2. Long-term selection: Crops, animals, and bacteria* (pp. 109–151). John Wiley & Sons. <u>https://doi.org/10.1002/9780470650288.ch4</u>
- Fanatico, A., & Ellis, K. (2016). Organic poultry production: Providing adequate methionine. ATTRA Sustainable Agriculture, National Center for Appropriate Technology.

https://attra.ncat.org/publication/organic-poultry-production-providing-adequate-methionine/

- Frey, K. J. (1951). The interrelationships of proteins and amino acids in corn. *Cereal Chemistry, 28*, 123–132. Abstract available via Google Scholar in References (Frey) at <u>https://link.springer.com/article/10.1007/BF00277674</u>
- Goldstein, W. (2016). Bulletin 1. Partnerships between maize and bacteria for nitrogen efficiency and nitrogen fixation. Mandaamin Institute. https://www.mandaamin.org
- Goldstein, W. (2022). Testing N efficient, high methionine corn hybrids with organic farmers (Final report for project LNC 17-389).
 U.S. Department of Agriculture, Sustainable Agriculture Research and Education. https://projects.sare.org/sare_project/lnc17-389/
- Goldstein, W. A., & Cambardella, C. (2008, March 12–14). Organic maize, organic matter management, root health, and nitrogen. In U. Koepke & S. M. Sohn (Eds.), ISOFAR International Conference on Soil Fertility proceedings (pp. 64–80). Dankook University, Korea.
- Goldstein, W., Jaradat, A. A., Hurburgh, C., Pollak, L. M., & Goodman, M. M. (2019). Breeding maize under biodynamic-organic conditions for nutritional value and N efficiency/N2 fixation. *Open Agriculture*, 4(1), 322–345 <u>https://doi.org/10.1515/opag-2019-0030</u>
- Goldstein, W., Andrade Laborde, J. E., Meyer, P., Gulkirpik, E., & Toc, M. (2023). Testing the quality of corn that has been selected for organic poultry. eOrganic. <u>https://eorganic.org/node/35728</u>
- Goldstein, W. A., Pollak, L. M., Hurburgh, C., Levendoski, N., Jacob, J., Hardy, C., Haar, M., Montgomery, K., Carlson, S., & Sheaffer, C. (2008, March 12–14). Breeding maize with increased methionine content for organic farming in the USA. In U. Koepke & S. M. Sohn (Eds.), *ISOFAR International Symposium on Organic Agriculture proceedings* (pp. 262–275). Dankook University, Korea.
- Goldstein, W. A., Schmidt, W., Burger, H., Messmer, M., Pollak, L. M., Smith, M. E., Goodman, M. M., Kutka, F. J., & Pratt, R. C. (2012). Maize breeding and field testing for organic farmers. In E. T. Lammerts van Bueren & J. R. Myers (Eds.), Organic crop breeding (pp. 175–189). Wiley-Blackwell. <u>https://doi.org/10.1002/9781119945932.ch10</u>

- Goldstein, W., White, J., Mujjabi, C., Gulkirpic, E., Toc, M., Bohn, M., Andrade, J., Ugarte, C., Nunez, M., Karnes, J., Lotfi, A., Kingsley, K., Travis, M., Wander, M., Jaradat, A. A., Mohr, S., & organic farmers. (2020, February 27– March 29). Breeding and testing nitrogen efficient/fixing, corn with high methionine and carotenoid contents for organic farmers [Poster presentation]. Midwest Organic and Sustainable Educational Service Conference, La Crosse, WI, U.S.
- Hacket, B. (2021). Farmers trapped in unsustainable cycle by biotechnology, seed consolidation [Blog]. National Sustainable Agriculture Coalition. <u>https://sustainableagriculture.net/blog/farmers-trapped-in-unsustainable-cycle-by-biotechnology-seed-consolidation/</u>
- Hardy, C. L, Rippke, G. R., Hurburgh, C. R., & Goldstein, W. A. (2009). Rapid measurement of corn amino acids using near infrared whole grain analyzers. Iowa State University Extension. <u>https://www.extension.iastate.edu/grain/files/page/files/CornaminoacidmeasurementIGQI2008_01_09Compatibi</u> <u>lit.pdf</u>
- Holding, D. R, Otegui, M. S., Li, B., Meeley, R. B., Dam, T., Hunter, B. G., Jung, R., & Larkins, B. A. (2007). The maize *floury1* gene encodes a novel endoplasmic reticulum protein involved in zein protein body formation. *The Plant Cell*, 19(8), 2569–2582. <u>https://doi.org/10.1105/tpc.107.053538</u>
- Huffman, R. D., Abel, C. A., Pollak, L. M., Goldstein, W., Pratt, R., Smith, M., Montgomery, K., Grant, L., Edwards, J.
 W., & Scott. M. P. (2017). Combining ability and environmental investigation of maize testcrosses in diverse organic production systems. *Crop Science*, 58(1), 253–263. https://doi.org/10.2135/cropsci2017.06.0364
- Irizarry, I., & White, J. F. (2018). Bacillus amyloliquefaciens alters gene expression, ROS production, and lignin synthesis in cotton seedling roots. Journal of Applied Microbiology, 124(6), 1589–1603. <u>https://doi.org/10.1111/jam.13744</u>
- Jacob, J. P., Levendoski, N., & Goldstein, W. (2008). Inclusion of high methionine corn in organic pullet diets. *Journal of Applied Poultry Research*, 17(4), 440–445. <u>https://doi.org/10.3382/japr.2008-00005</u>
- Jaradat, A. A., & Goldstein, W. (2013). Diversity of maize kernels from a breeding program for protein quality: I. Physical, biochemical, nutrient, and color traits. *Crop Science*, 53(3), 956–976. <u>https://doi.org/10.2135/cropsci2012.07.0437</u>
- Jaradat, A. A., & Goldstein, W. (2014). Diversity of maize kernels from a breeding program for protein quality: II. Correlatively expressed functional amino acids. *Crop Science*, 54(6), 2639–2662. <u>https://doi.org/10.2135/cropsci2013.09.0615</u>
- Jaradat, A., & Goldstein, W. (2018). Diversity of maize kernels from a breeding program for protein quality: III. Ionome profiling. *Agronomy*, 8(2), Article 9. <u>https://doi.org/10.3390/agronomy8020009</u>
- Jones, Z. G. (2018). Identification of useful traditional and gametophytic germplasm for maize improvement [Doctoral dissertation, North Carolina State University]. NC State Theses and Dissertations, University Libraries. <u>https://repository.lib.ncsu.edu/handle/1840.20/34942</u>
- Kandel, S. L, Joubert, P. M., & Doty, S. L. (2017). Bacterial endophyte colonization and distribution within plants. *Microorganisms*, 5(4), Article 77. <u>https://doi.org/10.3390/microorganisms5040077</u>
- Kimmerer, R. W. (2013). Braiding sweetgrass: Indigenous wisdom, scientific knowledge and the teachings of plants. Milkweed Editions.
- Kowalski, D. (2017). Organic and non-GMO specialty grains: Assessing the impact and opportunity for growers (Issue brief). CoBANK Knowledge Exchange. <u>https://sso.cobank.com/documents/7714906/7715335/Specialty-Grains-Report-Jan2017.pdf/e19b6095-483d-2d01-e1a3-90a95b201a3b?t=1607541504183</u>
- Levendoski, N., & Goldstein, W. A. (2006). Alternatives to synthetic methionine feed trial [Poster presentation]. In International Federation of Organic Agriculture Movements [IFOAM] (Ed.), Proceedings of the 1st IFOAM International Conference on Animals in Organic Production: St. Paul, Minnesota, USA, August 2006. IFOAM Head Office.
- Lu, Y., Moran Lauter, A., Makkena, S., Scott, M. P., & Evans, M. M. (2020). Insights into the molecular control of crossincompatibility in *Zea mays. Plant Reproduction*, 33(3–4), 117–128. <u>https://doi.org/10.1007/s00497-020-00394-w</u>
- Luo, S., & Levine, R. L. (2009). Methionine in proteins defends against oxidative stress. *The FASEB Journal*, 23(2), 464–472. <u>https://doi.org/10.1096/fj.08-118414</u>
- Mastrodomenico, A. T., Haegele, J. W., Seebauer, J. R., & Below, F. (2018). Yield stability differs in commercial maize hybrids in response to changes in plant density, nitrogen fertility, and environment. *Crop Science*, *58*(1), 230–241. https://doi.org/10.2135/cropsci2017.06.0340

- McBride, W. D., & Greene, C. (2015, November 2). Despite profit potential, organic field crop acreage remains low. Amber Waves, U.S. Department of Agriculture, Economic Research Service. <u>https://www.ers.usda.gov/amber-waves/2015/november/despite-profit-potential-organic-field-crop-acreage-remains-low/</u>
- McEvoy, M. (2015, September 3). Memorandum to the National Organic Standards Board. U.S. Department of Agriculture, Agriculture Marketing Service. <u>https://www.ams.usda.gov/sites/default/files/media/NOSB%20Memo%20Response%20to%20Rec%20from%20</u> April%202015%20Meeting.pdf
- Micci A, Zhang, Q., Chang, X., Kingsley, K., Park, L., Chiaranunt, P., Strickland, R., Velazquez, F., Lindert, S., Elmore, M., Vines, P.L., Crane, S., Irizarry, I., Kowalski, K.P., Johnston-Monje, D., & White, J.F. (2022). Histochemical evidence for nitrogen-transfer endosymbiosis in non-photosynthetic cells of leaves and inflorescence bracts of angiosperms. *Biology*, 11(6), Article 876. <u>https://www.mdpi.com/journal/biology</u>. <u>https://doi.org/10.3390/biology11060876</u>
- National Agricultural Statistics Service. (2022). Certified organic survey 2021 summary. U.S. Department of Agriculture. https://www.nass.usda.gov/Surveys/Guide to NASS Surveys/Organic Production/
- Padilla García, J. M., Sánchez González, J. de J., Larios, L. de la C., Ruiz Corral, J. A., Parra, J. R., & Morales Rivera, M. M. (2012). Incompatibilidad gametofítica en las razas mexicanas de maíz [Gametophytic incompatibility in Mexican maize breeds]. Revista Mexicana de Ciencias Agrícolas, 3(3), 525–537. https://doi.org/10.29312/remexca.v3i3.1446
- Pollak, L. M. (2003). The history and success of the public-private project on germplasm enhancement of maize (GEM). *Advances in Agronomy*, 78, 45–87. <u>https://doi.org/10.1016/S0065-2113(02)78002-4</u>
- Ross, A. (2018). The Turkish infiltration of the U.S. organic grain market: How failed enforcement and ineffective regulations made the U.S. ripe for fraud and organized crime [White paper]. The Cornucopia Institute. https://www.foodfarmingsustainability.com/food-and-agriculture
- Tsai, C. Y., Dweikat, I., Huber, D. M., & Warren, H. L. (1992). Interrelationship of nitrogen nutrition with maize (Zea mays) grain yield, nitrogen use efficiency and grain quality. *Journal of the Science of Food and Agriculture*, 58(1), 1–8. <u>https://doi.org/10.1002/jsfa.2740580102</u>
- University of Illinois. (2020). Ears to the ground, Part 6: Breeding for feed quality. An interview with Walter Goldstein [Video]. YouTube. <u>https://youtu.be/uTtuieh1YWU?si=1qGnCvFaEzk-i-Z6</u>
- Verma, S. K., Kingsley, K., Bergen, M., English, C., Elmore, M., Kharwar, R. N., White, J. F. (2017). Bacterial endophytes from rice cut grass (*Leersia oryzoides* L.) increase growth, promote root gravitropic response, stimulate root hair formation, and protect rice seedlings from disease. *Plant and Soil*, 422(1–2), 223–238. https://doi.org/10.1007/s11104-017-3339-1
- Verma, S. K., Kingsley, K., Irizarry, I., Bergen, M., Kharwar, R. N., White, J. F. (2017). Seed vectored endophytic bacteria modulate development of rice seedlings. *Journal of Applied Microbiology*, 122(6), 1680–1691. <u>https://doi.org/10.1111/jam.13463</u>
- Verma, S., & White, J. F. (2018). Indigenous endophytic seed bacteria promote seedling development and defend against fungal disease in brown top millet (*Urochloa ramosa* L.). *Journal of Applied Microbiology*, 124(3), 764–778. <u>https://doi.org/10.1111/jam.13673</u>
- White, J. F., Chen, Q., Torres, M., Mattera, R., Irizarry, I., Tadych, M., & Bergen, M. (2015). Collaboration between grass seedlings and rhizobacteria to scavenge organic nitrogen in soils. *Annals of Botany, Plants*, 7, plu093. <u>https://doi.org/10.1093/aobpla/plu093</u>
- White, J. F., Kingsley, K. L., Butterworth, S., Brindisi, L., Gatei, J. W., Elmore, M. T., Verma, S. K., Yao, X., & Kowalski, K. P. (2019a). Seed-vectored microbes: Their roles in improving seedling fitness and competitor plant suppression. In S. K. Verma & J. F. White (Eds.), *Seed endophytes: Biology and biotechnology* (pp. 3–20). Springer. <u>https://doi.org/10.1007/978-3-030-10504-4_1</u>
- White, J. F., Kingsley, K. L., Verma, S. K., & Kowalski, K. P. (2018). Rhizophagy cycle: An oxidative process in plants for nutrient extraction from symbiotic microbes. *Microorganisms*, 6, Article 95. <u>https://doi.org/10.3390/microorganisms6030095</u>

- White, J. F., Kingsley, K. L., Zhang, Q., Verma, R., Obi, N., Dvinskikh, S., Elmore, M. T., Verma, S. K., Gond, S. K., & Kowalski, K. P. (2019b). Review: Endophytic microbes and their potential applications in crop management. *Pesticide Management Science*, 75(10), 2558–2565. <u>https://doi.org/10.1002/ps.5527</u>
- Wu, Y., & Messing, J. (2012). RNA interference can rebalance the nitrogen sink of maize seeds without losing hard endosperm. *PLoS ONE*, 7(2), Article e32850. <u>https://doi.org/10.1371/journal.pone.0032850</u>