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HOW MAIZE EVOLVES WHEN IT IS BRED UNDER BIODYNAMIC/ORGANIC CONDITIONS AND SELECTED FOR IMPROVED NUTRITIONAL VALUE AND NITROGEN EFFICIENCY.

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Abstract: A breeding program for maize for biodynamic/organic farmers has taken place in at the Mandaamin Institute. The program began in 1989 and entails summer nurseries in Wisconsin, USA and winter nurseries in Puerto Rico and Chile. Emphasis has been on improving adaptation, productivity, nutritional value, and nitrogen (N) efficiency/N_2 fixation. Methods include pedigree breeding , development of synthetic populations, and selection under N limited conditions. A co-evolutionary approach is taken to optimize interactions between the maize populations, the breeder, and associated microbes, under N limited biodynamic/organic environments . Results have been shifts in protein quality and better adaptation to N limited conditions. Emergent evolutionary processes a) increased the occurrence of mutants with soft grain possessing higher protein quality; b) improved chlorophyll content and N efficiency, and c) resulted in the appearance of densely branched rooting systems in the top layers of the soil for N efficient inbreds and hybrids. Grain yields for the best hybrids have been competitive with conventionally bred hybrids, especially under N limited conditions. Grain quality of the resulting hybrids averaged 16 % more protein, 30 % more methionine, and 16 % more lysine than for conventional hybrids. Under poultry feeding conditions where some synthetic methionine is fed, the monetary value of the grain was 14 % higher because it reduced the need for soymeal in feed. Outcomes of the program are populations, inbreds, and hybrids which are now in wider spread strip plot testing on organic farms in Wisconsin, Illinois, Indiana, and have entered initial commercial production in conjunction with Foundation Organic Seed (Onalaska, WI). We will also report results from ongoing farm trials that assess relationships between rooting, N uptake, N mineralization of organic matter, N_2 fixation, and protein production in grain.

Introduction: Modern breeding programs for maize focus on selection for uniform inbreds and single-cross hybrids that produce reliable, high yields under optimally fertilized, conventional conditions. Such programs utilize biotechnology (transgenes and dihaploid breeding), are carried out by few, large scale conglomerates, and result in farmer ready products with breeding history that is not transparent.

As maize is regarded as being a heavy feeder, large amounts of N fertilizer are applied during its breeding phase and for on-farm grain production. That fertilizer results in nitrate pollution of surface and ground water with and results in the production of nitrous oxide, a potent greenhouse gas. N fertilizer also causes the accumulation of cereal storage proteins that have low contents of essential amino acids thereby reducing nutritional value. Use of N fertilizer increases the nutritionally least valuable α and γ zein content of maize grain, makes kernels harder (more vitreous), and reduces digestibility and overall protein nutritional quality.

In contrast, organic farmers need cultivars that sync with lower input organic growing conditions and the demands and expectations of organic consumers and environmentalists. Furthermore, maize grain is a major component of poultry and dairy feed for organic farmers but because the amino acid composition of it protein is deficient, synthetic methionine and soymeal are added to feed in the USA to compensate. Organic poultry farmers especially need an alternative to the use of synthetic methionine as permission to use this input is being phased out. In this paper we outline a breeding program, carried out mainly under biodynamic/organic conditions, that addresses and helps resolve these problems. In harmony with biodynamic/organic attitudes the project presupposes that the plant, its associated microbes, and the breeders are interactive partners in a biologically creative process leading to enhanced adaptation. By shifting to selection under N limited conditions, the effort addresses the N fertilizer and methionine problem and increases the value of the maize for agriculture and farmers.

Material and methods: For 14 years the program bred open pollinated populations for a small clientele of organic farmers. Afterwards, listening sessions with farmers revealed that though they wanted improved adaptation to organic conditions and enhanced nutritional value, most of them also wanted the high yields associated with hybrids. Furthermore, organic seed companies only wanted to sell uniform, single cross hybrids. As a consequence, our program produces inbreds and hybrids using pedigree breeding as well as improved open pollinated, synthetic populations. Crosses are made between promising parents and their derivatives are grown and selected for multiple traits generally under N limited conditions while being inbred for 6 to 10 generations. Selection criteria include productivity, resistance to various diseases and pests, synchronicity of silking and pollen shed, grain maturity and dry down, standing ability/stay green, and traits associated with N efficiency/N2 fixation (chlorophyll content, isotope composition, protein content, exudate production on prop root system). Seed is selected for opacity using a light table and tested for methionine, lysine, and cysteine using HPLC and NIRS. Promising lines are also crossed with standard inbreds and tested for agronomic performance on multiple sites. Yield trials take place in various organic farms in various states on local sites and on other sites in combination with the US Testing Network, the University of Illinois, and University of Wisconsin. In addition, N efficiency is being evaluated in 2019 in strip trial grow outs on 8 sites in Wisconsin. This research includes a) N analysis of roots, stalks, and grain to construct N uptake budgets; b) evaluation of rooting systems; c) evaluation of N isotopes of tissues and soils in the root area and in the unplanted area between rows to assess the dynamics of N mineralization and N_2 fixation.

Results: Early results from the breeding program were documented in Goldstein et al. 2019. This included 1) Emergence of soft kernelled mutants and of improved N efficiency for inbreds and breeding lines. 2) Protein content and amino acid quality found with our hybrids relative to conventionally bred hybrids (see Table 1). 3) The discovery of putative N_2 fixing landraces ; 4) Disinfection for Fusarium decreased productivity of conventional hybrids but did not significantly affect the N efficient/fixing hybrids (Table 2). Inoculation increased protein production for the N efficient hybrids but did not affect the conventional hybrids. 5) N efficient inbreds developed by our program had enhanced branched root production in the surface layers of the topsoil while conventional inbreds had more vertically oriented roots with less branching (Diagram

1). 6) The cultivars bred under N limited conditions outyielded the conventional hybrids under low N conditions (Table 3). 7) Several N efficient/putative N_2 fixing inbreds were developed.

Table 1. Protein and its quality of Mandaamin vs. conventional hybrids (after Goldstein et al., 2019).

Variable	HPLC (3 Conv. vs. 6 Mandaamin Hybrids)			NIRS (149 Conv., vs. 1250 Mandaamin Hybrids)		
	Conventional	Mandaamin	Diff %.	Conventional	Mandaamin	Diff. %
Protein (%)	7.93	10.50	32	8.62	10.0	16
Lysine (%)	0.290	0.331	14			
Methionine (%)	0.174	0.262	51	0.21	0.274	30
Cysteine (%)	0.174	0.215	24	2.46	2.76	12
Meth. In protein (%)	2.15	2.46	14			

Diagram 1 shows visual differences associated with the discovery of the C4 inbred family which is strongly N efficient/putative N2 fixing. After Goldstein, et al., 2019.

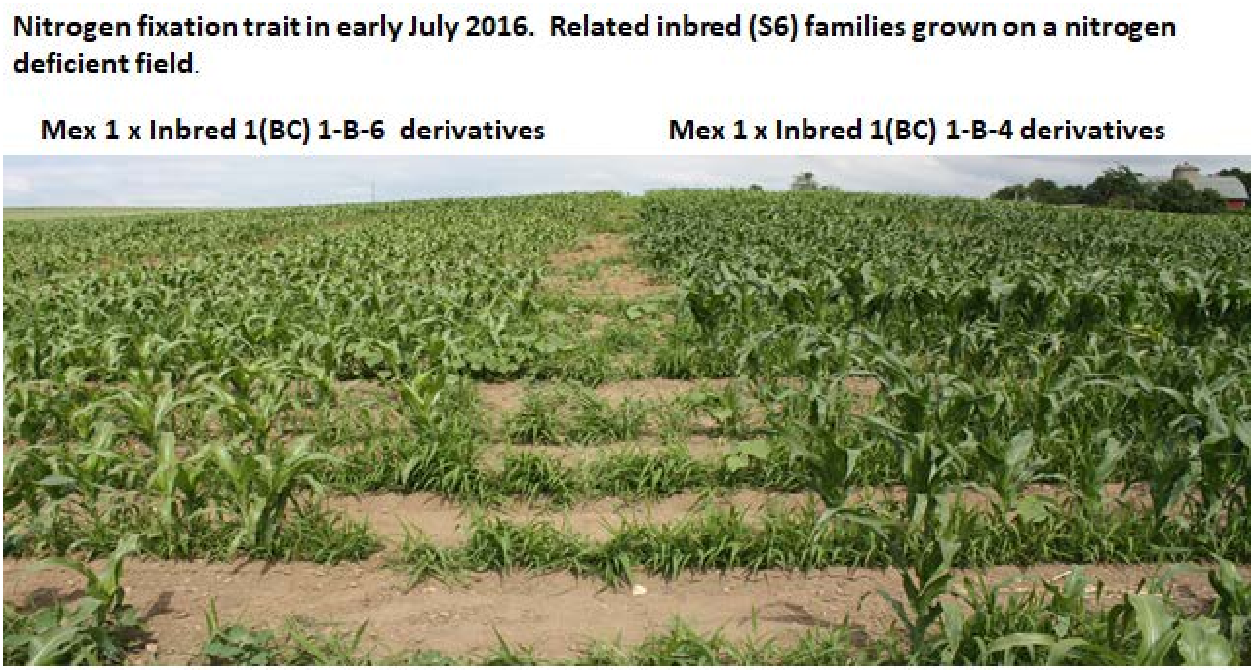

Table 2. Seed of conventional hybrids and new N efficient hybrids were pre-treated with and without disinfection to control Fusarium endophytes and with and without inoculation with N_2 fixing bacteria (after Goldstein et al., 2019).

Treatment	Effect	Plants/ha		Protein/ha	
		Difference (%)	p	Difference (%)	p
Disinfection on Conventional	Neg.	**37**	0.0001	**38**	0.0001
Disinfection on N-Efficient	Neg.	4	0.38	11	0.26
Inoculation on Conventional	Neg.	1	0.88	3	0.77
Inoculation on N-Efficient	**Pos.**	3	0.50	20	0.07
N-Efficient vs. Conventional w/o disinfection	**Pos.**	4	0.38	16	0.06
N-Efficient vs. Conventional w/o Inoculation	**Pos.**	21	0.001	20	0.06
N-Efficient vs. Conventional w/ Inoculation	**Pos.**	25	0.0001	35	0.0001

Diagram 1. Roots excavated from topsoils on two N limited sites in 2016. After Goldstein, et al., 2019.

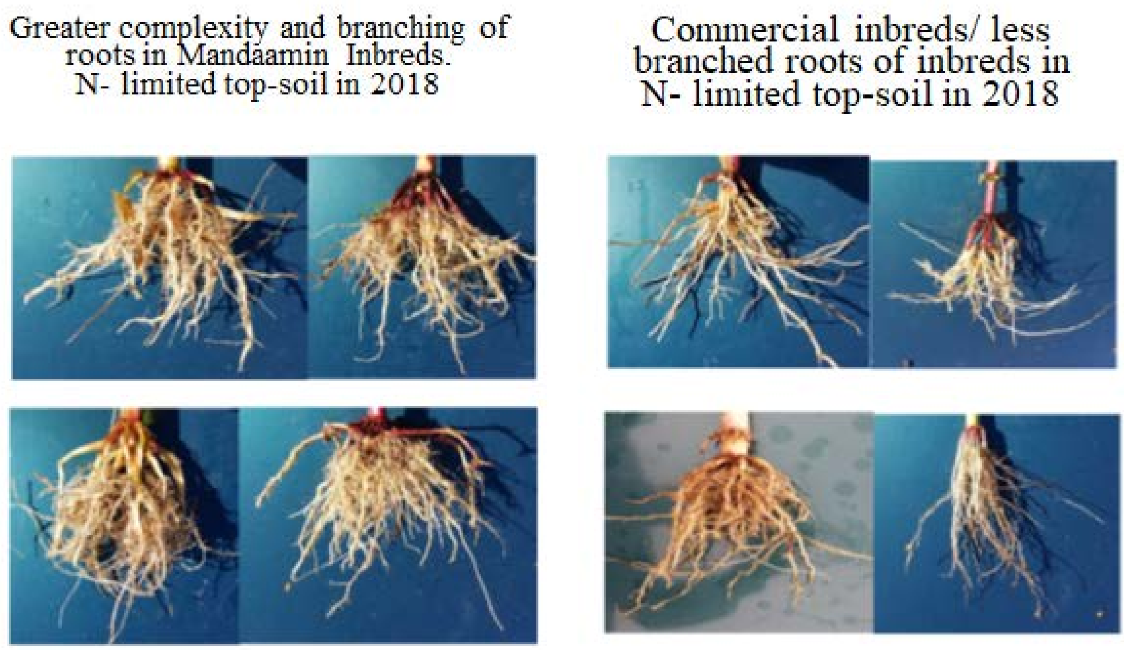

Table 3. Trials on three sites in 2016 to evaluate the effects of low fertility & low fertilizer rate on protein and its quality.

Site/Pedigree	GY t/ha	Protein %	Lysine %	Methionine %	Protein kg/ha
JR3 (low fertilizers)					
Mandaamin (5)	7.85	9.00	0.36	0.26	600
Conventional (4)	7.22	6.97	0.30	0.19	427
% diff (Checks)	**9**	**29**	**18**	**35**	**40**
BP (low fertilizers)					
Mandaamin (5)	9.42	8.88	0.36	0.27	703
Conventional (1)	7.78	6.96	0.29	0.19	461
% diff (Checks)	**21**	**28**	**20**	**38**	**53**
Zinn (manure)					
Mandaamin	11.17	11.28	0.39	0.30	1,066
Conventional	11.30	10.40	0.33	0.25	992
% diff (Checks)	**-1**	**8**	**19**	**23**	**7**

New information will be presented on a range of hybrids grown on strip trials on organic and conventional sites in 2019. Furthermore, observations in 2019: a) confirm that the putative N efficient/N_2 fixing inbreds possess densely branched rooting systems and that rhizophagy is actively occurring in root tips. Results suggest that as N efficiency increases, communities of microbes shift with reductions in Fusarium symptoms in plants and increases in Ustilago lesions in leaves.

Discussion: The partner model for breeding has been fruitful, resulting in high methionine cultivars that are N efficient. Emergent changes in plant performance under our environments are probably associated with shifts in epigenetic and genetic regulation and in fungal and bacterial communities associated with maize plants and living in or on them. Results and relationships between N uptake, root growth, N mineralization from organic matter, and N_2 fixation will be discussed.

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