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Breeding corn for organic farmers with improved N efficiency/N fixation, and protein quality.

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Abstract

The Mandaamin Institute breeds corn for organic farmers using classical breeding methods. Objectives include better N efficiency/N fixation, adaptation to organic soil conditions and better protein quality. Multi-state and year trials suggest that some of the new hybrids from the program may be competitive with commercial hybrids for grain yields while producing higher yields of protein. Naturally occurring N efficiency is being enhanced by crossing with landraces that demonstrated N efficiency/N fixation and through selecting inbreds on N limited sites. These are multiple races from highland Mexico, upland and lowland South America, and the Southwestern USA. Trials compared these landraces and their crosses with Corn Belt adapted cultivars under N limiting conditions over several years. The growth response of the landraces or their derivatives and the levels of natural N isotopes in their tissues indicated they had greater N efficiency, better ear set, and in some cases had substantial N fixation by associated diazotrophic bacteria. Plants with a larger tissue N pool may produce high grain protein contents more reliably. In search for grain quality, high methionine, opaque kernels emerged in multiple populations, lines, and inbreds over time. There was a spontaneous pattern of emergence of opaque kernelled phenotypes, lack of clear Mendelian patterns of segregation, and difficulties in fixing the trait in some backgrounds. Selection under organic growing conditions may induce shifts in epigenetic control of zein protein production in grain, reversing consequences of breeding for cultivars that respond to N fertilization.

Introduction

From an evolutionary and economic perspective it makes sense to select cultivars under the target environments under which they will be grown in order to foster adaptation. Though little is known about how corn bred under conventional conditions adapts to organic conditions, it is commonly recognized that hybrids developed under conventional conditions can produce high yields under organic conditions. But aside from yield, it is probable that corn for organic farms might need traits that conventional corn does not possess. Organic farmers want varieties that can prevent contamination from GMO pollen, have high nutritional value, and produce reliably well under organic conditions. Organic conditions entail that the varieties should be able to obtain sufficient N without synthetic fertilizers, and compete well with weeds without herbicides (Goldstein et al. 2012).

Corn with high methionine content is important for organic poultry producers as methionine is a major limiting amino acid (Goldstein et al, 2012). Though organic farmers are allowed to use synthetic methionine, its use is restricted, with possible phase-out in the coming years [77 Federal Register 57985]. Robust hybrids and inbreds that compete well with weeds are also important for organic hybrid production and for seed production.

Breeding Philosophy and Objectives

The Mandaamin selection program intends to develop adapted, robust, weed-competitive cultivars with 1) higher protein content, protein quality, and carotenoid content; 2) greater N efficiency; 3) cross (gametophytic) incompatibility with transgenic cultivars to avoid pollen contamination from GMO’s; and 4) competitive yields and low grain moisture contents at harvest. Organic farmers participate in the program by providing advice, growing environments for yield trials and nurseries, grow-outs of selected

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breeding stocks, and in some cases by selecting populations. The following narrative will focus on our results with N efficiency, protein production, and protein quality.

Methods

Mandaamin Institute used a standard inbreeding-based selection program coupled with early generation testing of grain yields. Occasionally, Mandaamin recombines selected inbreds to make improved synthetic populations, from which inbreeding and selection occurred. Breeding took place on nearby organic farms. Mandaamin systematically tested inbreds for N efficiency on field sites that are low in available N. An organic winter nursery at the University of Puerto Rico also enables Mandaamin to have two breeding seasons each year.

Crosses for hybrid yield trials were generally made starting at the S2 or S3 stage of inbreeding by crossing breeding lines with inbred testers from an opposite heterotic group. Replicated yield trials of 100 to 1000 hybrid entries took place on two to four organic sites in Iowa (in conjunction with Dr. Jode Edwards, USDA-ARS)and one site in Wisconsin in 2011, 2012, and 2013. Yield and moisture data obtained from these trials are critical for deciding which inbreds to continue to inbreed with selection pressure generally ranging from 2 to 15% depending on the results. The best 10 to 25 hybrid selections from a testing cycle are subsequently submitted to yield trials in comparison with commercial hybrids and hybrids from other breeding programs managed by the U.S. Testing Network (USTN, Ames, Iowa). USTN trials have taken place in three growing zones (100, 105 and 112 day relative maturity) on organic sites in New York, Pennsylvania, Ohio, Indiana, Illinois, Iowa, Wisconsin, and Nebraska.

Selection for N efficiency/N fixation of breeding lines occurred under N limiting conditions. Plants are grown on soils with low organic matter content, or under N immobilizing conditions caused by grass thatch buildup or growing corn after corn. Seed was inoculated with a mixture of diazotrophic bacteria that have been isolated from the roots, rhizosphere, rhizoplane, and internal tissues of corn (formulated by Terra-Max, Inc., Bloomington, MN).. Inbred lines grown under these conditions were evaluated in early June for emergence, color, and height. Values for each of these parameters are converted into a fraction of the mean overall value, which are averaged for a given breeding line. Only lines that are above the overall mean are self-pollinated and lines with unusual disease or insect infestations are avoided. Methionine, lysine, and cysteine content of whole grain were determined by a a near infra-red spectroscopic (NIRS) calibration (Jaradat and Goldstein, 2013).

Results

Yield: A few of the hybrids derived from Mandaamin x Mandaamin or Mandaamin x commercial inbreds appear to be yield competitive and have moisture contents similar to commercial organic hybrids in multi-year and site trials. Where protein data was available, protein yields per acre are often considerably higher than for conventional hybrids.

Nitrogen Efficiency/Fixation: In 2009 we found exotic corn landraces that were N sufficient on a N limiting site relative to deficient corn belt corn varieties. Analysis of the stable δ-15N isotopes from grain samples suggested that some of the exotic cultivars fixed up to half of their N from the air. The landraces that showed N efficiency/N fixation and are being used in our breeding program were introduced from highland Mexico, including Azul, Gordo, Cristalino de Chihuahua, Cacahuacintle, Celaya, Conico, and Mixeno; from upland and lowland South America; and from the Southwestern USA. Hybrid crosses with selected exotics produced approximately 40% more protein, lysine, methionine, and cysteine per acre than did conventional hybrids ($p < 0.001$) on an unfertilized site in 2010. Inoculation of seed with a mix of N fixing bacteria increased yields of grain and protein for the exotic crosses by 18 and 19%, respectively ($p<5\%$) but did not affect conventional hybrids (Goldstein, 2012b). We bred families and lines from these hybrids, inoculated their seed with bacteria and grew them in a replicated trial on N-deficient, P and K and moisture sufficient, sandy soils in 2013. The two conventionally bred check populations averaged 77% barren plants. In contrast the top 20% of N efficient lines had 10 to 40% barren plants.

Some of the breeding lines that have been derived from crosses with these races and are putative N efficient/N fixing show very dark green foliage indicating a high N content when grown on multiple N deficient sites. They show earlier flowering, and superior ear set under low N and drought stress conditions than sister lines that do not possess the trait. Expression of the trait in breeding progeny is variable. We are doing field trials to establish which of the lines have yield potential in order to produce commercial grade hybrids for seed companies, and we are backcrossing the trait into adapted cultivars.

High methionine corn and the emergence of opaque kernels: In earlier work we had developed high methionine, lysine, and cysteine corn based on floury-2 (fl2) allele. Initial feeding trials (Organic Valley and University of Minnesota) suggested that high methionine fl2 corn (approximately 0.3% methionine) might replace synthetic methionine for organic poultry (Jacob et al, 2008; Levendoski and Goldstein, 2006), confirming earlier studies (Cromwell et al, 1968; Chi and Speers, 1973). However, yields were inadequate, in part due to the pleiotropic effect of the allele, conditioning a smaller seed size, confirming similar indications in the scientific literature (Lorenzoni et al, 1980; Coleman et al., 1995). Therefore, we searched through our non fl2, hard endosperm breeding families for opaque seed, and used NIRS to screen for high protein, methionine, cysteine, and lysine.

We found a new set of opaque kernelled selections in a wide set of 13 breeding families, mostly derived from GEM crosses of normal, translucent-kernelled landraces with normal commercial inbreds. These kernels had little seed size and yield reduction and had high nutritional quality. Their pedigrees and quality were documented in two papers in Crop Science (Jaradat and Goldstein, 2013 and 2014; Goldstein, 2012a).

In 2011 we had wet chemistry done by the University of Missouri Chemical Testing Lab on six of our new floury hybrids. They averaged 10.9% protein, 0.25% methionine. Eight conventional hybrids averaged 8.1% protein and 0.17% methionine. Qualitative gel electrophoresis (conducted by Dr. David Holding, University of Nebraska) suggested several of our non floury-2 breeding lines, from different backgrounds, had reduced α zeins but increased β and δ zeins and non-zein contents with better nutritional value.

The emergence of soft, opaque kernels out of translucent populations was noticed first in 2006 from our populations which had hitherto been selected for hard kernels and high test weight and from GEM derived populations derived from crosses of translucent kernelled parents. Opaque kernels began to appear in unexpectedly large amounts in families in which there was little or no opaque seed in previous generations. Studies with numerous families did not indicate that the trait segregated in any kind of clear Mendelian pattern. In some backgrounds it proved difficult to fix the trait. Subsequently, there has been continued spontaneous occurrence of opaque kernels in populations, breeding lines, and in inbreds. Crossing tests and kernel weight differences between translucent and opaque kernels suggest that the trait is not fl2.

Discussion

The Mandaamin program is producing phenotypes with better N efficiency and quality protein. N efficiency has been increased by crossing Corn Belt inbreds with landraces that respond to inoculation with diazotrophic endophytes and perform relatively well on N limited soils. Several of the upland landraces from Mexico we have tested have the trait, and it probably is widespread and present in many other Mexican landraces that we have not tested. We have found the trait in both lowland and highland races from South America, indicating that the trait is widespread and probably has been selected for under conditions of native farming. Comparisons of advanced sister breeding lines that possess or do not possess the trait showed that the trait is associated with earlier flowering, and better ear set under drought and N stress conditions. Possession of the trait reflects profound physiological shifts that may result in a better adapted plant with a larger tissue N pool available for producing reliably high grain protein contents. We are in process of finishing inbreds, testing hybrid combinations in yield trials, and backcrossing the trait into adapted inbreds in order to make it commercially available.

In our program we are probably witnessing a reversion of the genetic and epigenetic patterns induced by N fertilization. Low N-input organic farming may be conducive to the expression of higher quality protein, and conversely, selection for high yielding cultivars under high soluble N, conventional farming probably induces poor quality protein. We hypothesize that the opaque 'emergence' found in our program is due to general epigenetic shifts in zein production, induced by low N conditions (Mueller et al, 1997), shifting microbial relationships, by our selection processes, or some combination thereof.

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