Original Paper **Open Access**

NDSU EarlyGEM: Incorporating Tropical and Temperate Elite Exotic Germplasm to Increase the Genetic Diversity of Short-Season Maize

Santosh Sharma, Marcelo J Carena*

Dept. of Plant Sciences, North Dakota State Univ., Dep. #7670, Fargo, ND 58108-6050
*Corresponding author: E-mail: marcelo.carena@ndsu.edu

Abstract

The NDSU EarlyGEM or the Early Germplasm Enhancement of Maize (*Zea mays* L) is a long-term incorporation program designed to increase the genetic diversity of US northern maize hybrids. Exotic GEM breeding crosses derived from two temperate accessions, three tropical accessions and a tropical hybrid along with temperate US lines B73, Mo17, and Iowa Stiff Stalk Synthetic (BSSS) used as checks, were adapted to short-seasons and incorporated via a modified backcross (BC) procedure. The objective of this research was to test whether exotic derived crosses could be quickly adapted and incorporated to increase the genetic diversity of early maturing maize hybrids as compared to temperate US check lines. Exotic testcrosses with testers belonging to opposite heterotic groups were tested in five North Dakota environments and two years (2009 and 2010). Among 236 experimental testcrosses, 64 were statistically not different (LSD, 0.05) to industry hybrids for grain yield. BC derived lines from BR52051, CHO5015, DKB844 produced hybrids with low grain moisture at harvest (<87 RM) and high yield as compare to US lines. The derived lines of accessions SCRO1, BR52051, CHO5015 and CUBA117 produced hybrids with high grain oil (49 g kg^{-1} vs. 41 g kg^{-1}) and grain protein (102 g kg^{-1} vs. 91 g kg^{-1}) contents compared to checks. These results clearly showed that the exotic incorporations are the sources of unique new alleles for adaptation, yield, and quality traits for early maturing maize not present in existing US commercial germplasms and genome sequences (e.g. B73, Mo17, and BSSS). The NDSU EarlyGEM program is a source of unique and elite alleles and products.

Keywords: germplasm enhancement, adaptation, maize, grain, plant genetic resources

Introduction

North Dakota (ND) acreage of maize (*Zea mays* L) had record increases from 590,000 acres of maize planted in 1997 to 2.1 million acres planted in 2010 with a record 2.5 million acres planted in 2007. One of the reasons for this significant increase is the renewable fuel demand (Carena et al, 2009) and the change in weather patterns favoring maize production (Ransom et al, 2004). In ND, maize is expanding northward and westward to areas previously considered marginal for maize production. However, this process has been slow due to the lack of new short-season products and industry investment. Industry hybrids are still late maturing, lack stress tolerance, are slow driers, and often end up with poor quality. One of the main reasons is that ND commercial hybrids are mostly bred elsewhere (eg. southern MN) and retailer companies license products from Foundation Seed Companies located far from target areas, making their adaptation to short-seasons challenging. In addition, intellectual property and the very confidential maize business have limited breeding access to improve the few elite commercial lines available. The GEM program is an example on how ideal hybrids could be produced if breeding access is available.

Narrow genetic base of US germplasm sources (Goodman, 1990; Mikel and Dudley, 2006; Troyer, 2009) in maize could have affected the breeding process as there are very few alternatives for fast dry down products except for few Iodent and Minnesota 13 derived lines (Goodman, 2005) as well as NDSU derived lines (Carena et al, 2009). In ND, maize often needs to be harvested at moisture levels too high for safe storage and must be artificially dried for storage and transport (Yang et al, 2010).

Improved maize exotic germplasms represent potential sources of significant genetic improvement and of new alleles different from B73, and other elite lines and germplasms recently sequenced (Hallauer and Carena, 2009). The utilization of exotic germplasms has been reported to be useful to increase the genetic diversity of the crops for various important traits including many biotic and abiotic stresses (Hallauer, 1978; Goodman, 1999). Tropical and temperate exotic germplasms were found to have potential for yield improvement in US temperate hybrids (Goodman et al, 1990; Uhr and Goodman, 1995; Goodman, 1999; Tarter et al, 2003). Higher genetic diversity for several quality traits was reported in exotic maize germplasms (Milton, 1971; Singh et al, 2001; Pollak, 2003).

Exotic germplasm incorporation is a unique way

 RECEIVED 11/23/2011

to genetically diversify breeding germplasms through favorable and unique alleles (Simmonds, 1993). Learning to adapt new crop varieties to changing climates can ensure food security and political stability (CSSA, 2011). The NDSU EarlyGEM (the Early Germplasm Enhancement of Maize Program) is a long-term incorporation program initiated in 1999 to increase the genetic diversity of short-season abiotic stress tolerant hybrids. It utilizes the GEM project as an intermediate adaptation process to incorporate diverse and unique alleles from tropical and temperate exotic germplasms. Diverse and unique exotic breeding crosses were incorporated through a series of nursery observations and backcrossing to adapted lines. As a consequence, thousands of BC1: S1 lines were produced for evaluations (Carena et al, 2009). The GEM project followed the Latin American Maize Project (LAMP). LAMP project evaluated 12,000 maize accessions from 34 regions of Latin America and US. This was the biggest event in the history of maize germplasm evaluation including 74% known maize races (Sevilla and Salhuana, 1997).

Backcrossing has been shown to be an effective procedure to transfer traits controlled by multiple genes (Briggs and Allard, 1953). If one of the parents has more loci containing favorable alleles than the other, the first backcross is a superior method in population development that can be used for selection (Dudley, 1982).

The purpose of this research was to establish a long-term procedure to increase the genetic diversity of short-season hybrids with unique and elite exotic germplasm in a cost-efficient way. Recent researches have found higher genetic variation reported for flowering time and plant height in tropical exotic as compared to US temperate germplasm sources (Buckler et al, 2009; Coles et al, 2010; 2011). The application of our research intends to validate this basic idea through research and the development of unique products.

Materials and Methods

The experimental materials initially were evaluated by the LAMP project for drought tolerance, disease and insect resistance to northern leaf blight (*Exserohilum turcicum*), army worm (*Spodoptera frugiperda*), ear worm (*Heliothis zea*) and Fusarium ear rot (*Fusarium moniliforme*), cold tolerance, and grain quality traits after extensive evaluation of 12,000 maize accessions in 12 different countries (Sevilla and Salhuana, 1997). The GEM project started with the selection of a set of 23 tropical accessions identified by the LAMP project. The accessions were originally from Brazil, Mexico, Cuba, Barbados, British Virgin Islands, Dominican Republic, Puerto Rico, St. Croix, Antigua, Guatemala, and Peru. The second set consisted of seven tropical hybrids from a seed company 'DeKalb'. The third set included 27 accessions selected from temperate environments in Argentina, Chile, Uruguay and the USA. The accessions were assigned in groups of four to a total of 21 companies to make crosses with their tropical elite inbred lines. These materials were then assigned to different companies to make crosses to a second adapted inbred line (Salhuana, 1997).

In 2001, 152 GEM S_3 lines from released GEM central US Corn Belt sets A, B, and C derived from breeding crosses adapted to the US Central Corn Belt were obtained from Ames, Iowa. Three sets were selected. Set A was selected based on 1997 and 2000 yield trials. Set B lines were selected based on 1998 and 2001 yield trial tests. Set C lines were selected based on 1999 and 2002 yield trial tests (http://www.public.iastate.edu/~usda-gem/ assessed May 4, 2011). The lines were observed for 15 adaptation traits in ND short season nursery. The best 28 (<20% of all GEM lines evaluated) adapted lines (based on earliness and agronomic data in Fargo, ND) and top yielding genotypes (based on central US Corn Belt GEM trials) were selected and crossed to ND inbred line ND2000. ND inbred line ND2000 was used as recurrent parent to produce BC_1:S_0 source populations. Photoperiod conversion to the short-season of ND was carried out by selecting the earliest flowering plants among the segregating individuals per populations. Visual selection was used to discard late lines with agronomic deficiencies (poor stands, low seedlings vigor under cold stress, drought stress, lodging, insect and disease susceptibility, and height). Only nine populations were kept to produce BC_1:S_1 elite

Table 1 - GEM breeding crosses utilized as source of NDSU EarlyGEM unique lines.

Pedigree	Name	Race	Country	Ecological Adaptation	References
DKB844:S1601-507-1-B-B	GEM 10	Hybrid-tropical	Mexico	Tropical Exotic	(GEM, 2011)
CUBA 117:S1520-388-1-B	GEM 3	Argentino	Cuba	Tropical Exotic	(GEM, 2011)
BR52051:N04-70-1	GEM 5	Dente Amarelo	Brazil	Temperate Exotic	(GEM, 2011)
SCR01:N1310-265-1-B-B	GEM 4	St. Croix	St. Croix	Tropical Exotic	(GEM, 2011)
FS8B(T): N1802-35-1-B-B	GEM26	Mixed	USA	Tropical Exotic	(GEM, 2011)
CHO5015:N12-123-1-B-B	GEM22	Camelia	Chile	Temperate Exotic	(GEM, 2011)
BSSS(HT)C5	B73		USA	Temperate	(Russell, 1970)
C103 x187-2	Mo17		USA	Temperate	(Zuber, 1973)
16 SS lines	BSSS		USA	Temperate	(Troyer , 1999)

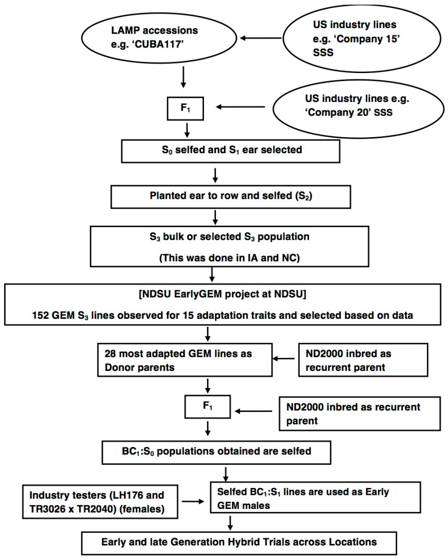

Figure 1 - NDSU EarlyGEM protocol (adapted from Hallauer et al, 2010)

12.5 % if we also consider industry germplasm used in original GEM crosses) (Figure 1). The recurrent parent ND2000 is a yellow-dent maize NDSU inbred line released in 2002 (Carena and Wanner, 2003). It has produced early maturing hybrids with higher grain yield, low grain moisture at harvest, high test weight, and very good stalk and root lodging resistance in the northern US Corn Belt.

Testcrosses

Over 200 early maturing lines developed from nine unique BC populations (Table 1) were testcrossed to industry testers. These included the $BC_1:S_1$ lines derived from early versions of six GEM breeding crosses and BC check populations including B73, Mo17, and Iowa Stiff Stalk Synthetic (BSSS) as donors.

Useful genetic diversity was evaluated with testers belonging to opposite heterotic groups. LH176 is an inbred industry tester representing the non-SS heterotic group (NSS) derived from LH82, P3704 (MBS Genetics, 2010). This was crossed to BC early generation lines from GEM10, GEM3, BSSS, B73 representing the BSSS heterotic group (SS). TR3026 x TR2040, is a sister line industry tester representing SSS. The TR3026 was derived from B14, B73 and TR2040 was derived from B14 (MBS Genetics, 2008). This was crossed to lines from GEM26, GEM22, GEM4, GEM5, and Mo17 representing the NSS heterotic group. To represent the diversity present in each of the $BC_1:S_1$ lines a maximum number of males plants (in average 8-10) were selected for crossing to female testers within each row.

Yield Trials

The progeny produced from each female row was shelled in bulk and the seeds were the source for testing in multi-location trials across ND environments. Trials were conducted with testcross hybrids from each tester as a single experiment for a total of two experiments per location. Experiments were arranged in 12 x 12 partially balanced lattice designs with two replicates in each location for NSS groups of lines. Experiments were arranged in 10 x 10 partially balanced lattice designs with two replicates in each location for the SSS groups of lines. In 2009 both experiments were placed in four locations of ND (Barney, Casselton, Prosper and Larimore) but due to very short and cool growing season only Prosper and Casselton for testcrosses with SSS group of lines and Prosper, Casselton and Barney for NSS group of lines were harvested. In 2010, the SSS testcrosses were placed in three locations (Casselton, Prosper and Larimore) and the NSS testcrosses were placed in four locations (Prosper, Casselton, Barney and Larimore). In 2010, all locations were harvested. Flowering notes were taken only in two locations (Fargo and Casselton) for both years. Four popular top performing industry hybrids representing the 83-93RM maturity range were used as checks (83RM DKC33-54, 87RM Pioneer 39D85, and two 90RM ad 92 RM BASF-Thurston Genetics hybrids). Detailed environmental notes were taken in each of germination, after flowering and before harvesting time. Each plot of 7 m long row and 0.76 m between rows was planted with 45 seeds which were thinned to 40 plants per plot at the four leaf stage to maintain the population size of 86,110 plants per hectare. Data on days to anthesis was noted when at least 50% of the plants in the plot were shedding pollen with at least 50% of the anthers emerged. Days to silking was noted when at least 50% of the plant in the plot were displaying visible silks. Mean plant height (cm) at maturity was measured as height from the ground to the terminal node of ten competitive plants per plot. Mean ear height (cm) was measured as height from the ground to node of uppermost ears in the same sample utilized for plant height. Root lodging (%) was measured by the percentage of plants leaning greater than 30° from vertical with intact stalks. Stalk lodging (%) was measured as the percentage of plants broken below the ears. Grain moisture at harvest (g kg^{-1}) was measured by a moisture blade in the combine harvester. Grain yield (Mg ha^{-1}) was obtained through the collection of plot wet weights which was transferred from pound per plot to Mg ha^{-1}. A sub-sample of 500 g of kernels was collected from every plot from all environments and used to measure the grain quality of all the genotypes. Grain quality screening was conducted in a Infratec© 1241 Grain NIR (Near Infrared Reflectance) analyzer in cooperation with Monsanto company. The equipment measured protein, oil, starch, extractable starch (HES), and fermentable starch (HFC) contents of maize grains.

Statistical Analysis

Table 2 - Top 17 testcross means¶ among SSS † lines with low moisture and high yield across six ND environments in 2009 and 2010.

Entries	H_2O at Harvest	Grain Yield#	Stalk Lodge	Root Lodge	Ear ‡ Height	Plant ‡ Height	Anthesis‡ Date	Silk ‡ Date
	(g kg^{-1})	(Mg ha^{-1})	(%)	(%)	(cm)	(cm)	D §	D §
LH176 x[(Gem 10xND2000)xND2000-1]-42	226	7.8	0.0	0.0	89.5	194.8	66	67
LH176 x[(Gem 10xND2000)xND2000-1]-31	225	7.2	0.7	0.0	96.8	198.2	67	68
LH176 x[(B73 x ND2000)xND2000-1]-45	246	7.1	0.0	0.0	86.3	192.0	66	67
LH176 x[(Gem 3xND2000)xND2000-1]-81	246	7.1	1.1	0.0	95.7	202.1	68	69
LH176 x[(Gem 10xND2000)xND2000-1]-33	246	7.1	1.1	0.0	94.7	196.6	68	69
LH176 x[(Gem 10xND2000)xND2000-1]-17	235	7.1	1.2	0.0	89.3	197.5	66	68
LH176 x[(B73 x ND2000)xND2000-1]-68	251	7.0	0.0	0.8	87.9	192.6	66	67
LH176 x[(B73 x ND2000)xND2000-1]-12	242	7.0	0.3	0.0	89.1	193.6	68	69
LH176 x[(Gem 3xND2000)xND2000-1]-54	244	7.0	0.4	0.0	88.2	191.6	68	69
LH176 x[(Gem 10xND2000)xND2000-1]-9	239	6.9	2.2	0.0	94.0	199.2	68	69
LH176 x[(Gem 10xND2000)xND2000-1]-22	226	6.9	0.8	0.7	93.2	199.6	67	68
LH176 x[(Gem 3xND2000)xND2000-1]-30	236	6.8	0.0	0.0	86.1	192.2	67	68
LH176 x[(Gem 10xND2000)xND2000-1]-23	220	6.8	0.0	0.4	92.9	199.9	67	69
LH176 x[(B73 x ND2000)xND2000-1]-70	234	6.8	0.0	0.0	85.3	187.7	65	66
LH176 x[(Gem 10xND2000)xND2000-1]-47	248	6.8	0.0	0.0	86.2	194.2	67	69
LH176 x[(Gem 3xND2000)xND2000-1]-97	234	6.7	0.4	0.0	96.3	203.7	67	69
LH176 x[(Gem 10xND2000)xND2000-1]-39	242	6.7	0.4	0.4	96.4	200.8	67	69
NP2623CBLL x TR3030	258	8.5	0.3	0.7	107.2	214.3	70	71
TR3127GT x TR3621CBLLRW	290	8.2	0.0	0.3	92.7	210.6	69	70
Pioneer 39D85	224	7.5	2.9	0.0	97.0	204.5	67	68
DKC33-54	194	7.1	0.0	0.3	82.0	193.4	65	66
Experimental Mean	243	6.4	0.7	0.2	90.9	195.5	67	68
LSD 0.05	23	1.8	2.2	1.2	6.8	9.0	2	2

†Stiff Stalk Synthetic heterotic group
‡Significant G x E interaction at 0.05 level of significance.
§ Days after planting.
¶ Means adjusted for lattice effects.
# Grain yield adjusted for 155 gm of water (H_2O) per kilograms of grain.

Data from each of the environments were analyzed by SAS version 9.2 (SAS Institute, 2008). PROC MIXED procedure with default REML in SAS was used to combine analyses using adjusted means for lattice effect from each traits and environments. Each location by year combination was considered as an environment. Entries (testcross entries and check entries) were considered as fixed and environment, replication (environment), block (replication x environment) and entry x environment were considered as random. The entry x environment mean square error was used as the error term. Fisher Protected Least Significant Differences (LSD) was used to compare the differences among genotype means at <0.05 level of significance. The mean of the testcrosses were considered as the means over environments adjusted for lattice effects. Entry x environment interaction error was used to calculate the LSD comparing entries means (checks and testcrosses). To determine correlation of rank of genotypes in different environments Spearman's coefficient of rank correlation was calculated by using PROC CORR with the SPEARMAN option in SAS. The phenotypic correlations of the different traits were calculated using Pearson's correlation using PROC CORR with the PEARSON option in SAS.

Results and Discussion

All the traits showed highly significant (P<0.01) differences across genotypes and five ND environments except root lodging and stalk lodging (P >0.05). The exotic testcross hybrids were not different than industry checks and the hybrids from temperate lines B73, Mo17 and BSSS for lodging resistance. It showed the incorporation process, adapted exotic lines to ND short seasons producing hybrids with minimal (below 3%) lodging as compared to their initial stage of adaptation (Tables 1 and 2). Lodging is one of the important problems with exotic germplasm, however, improvement of lodging in incorporation of tropical exotic germplasm was also reported by Holly and Goodman (1988) using visual selection in nursery.

Many early flowering lines were recovered from late exotic germplasms. There were 99 NSS lines and 31 SSS lines that were not different than 83 RM check DKC 33-54 for days of silking in hybrid combination with testers (Figures 2 and 3). Among them six hybrids were from Mo17 background and six and eight from BSSS and B73 genetic backgrounds respectively. The evidence showed the large standing variation in flowering time (Coles et al, 2010; 2011; Buckler et al, 2009) can be exploited for adaptation to early flowering with selection of earliest flower-

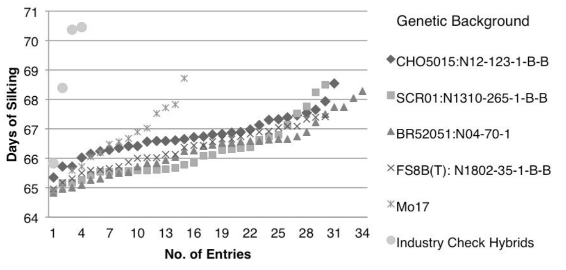

Figure 2 - Days of silking of maize testcross hybrids representing different NSS genetic backgrounds tested in four environments and two years [experimental mean was 68.3 days after planting (DAP) and LSD (0.05) was 1.9 DAP].

ing plants in a population by crossing with adapted parents and testing in unique short season environments. This evidence was especially important for lines derived from tropical exotic germplasms. Early flowering lines were recovered from late tropical incorporations including lines derived from FS8B (T), SCR01, CUBA117 and DKB844 (Figures 2 and 3).

The Pearson's correlation between grain moisture and days of silking was not large. Among NSS lines correlation was 0.53 (P<0.0001) and 0.48 (P<0.0001) among SSS lines for days of silking in hybrid combination with testers. Many of the lines derived from the incorporation were early flowering but also had slow dry down rate ending with high grain moisture at harvest. The results showed the importance of fast dry down when compared to early flowering in short-season environments which was also reported by Yang et al (2010) which should be addressed in basic science studies as well.

Fast dry down is an essential trait for northern US environments. High yielding hybrids are not successful in these environments unless grain moisture at harvest is adequate due to extra cost associated with drying. In NSS lines, 32 testcrosses were not different (LSD, 0.05) than the driest 83 RM check DKC33-54 for grain moisture at harvest. Backcross lines derived from Brazilian accession BR52051 and Chilean accession CHO5015 had produced many testcrosses with 30-60 g kg^{-1} less grain moisture at harvest as

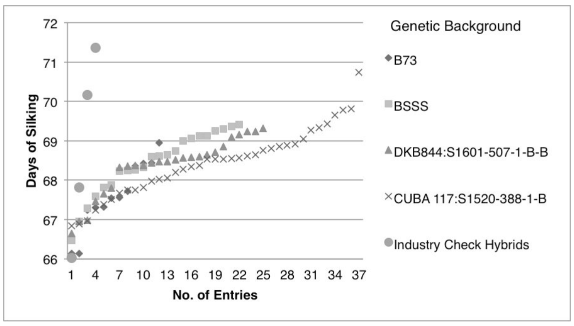

Figure 3 - Days of silking of maize testcross hybrids representing different SS genetic backgrounds tested in four environments and two years [experimental mean was 66.5 days after planting (DAP) and LSD (0.05) was 1.7 DAP].

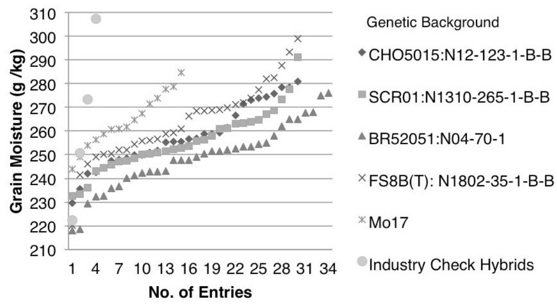

Figure 4 - Grain moisture content of maize testcross hybrids at harvest representing different NSS genetic backgrounds tested in five environments and two years (experimental mean was 258 g kg^{-1} and LSD (0.05) was 29 g kg^{-1}).

compared to NSS check parent Mo17 (Figure 4). Many of the lines derived from tropical accessions were similar to the grain moisture of 87 RM check hybrid Pioneer 39D85 showing their adaptation to regions that need 87RM and below. Within the SS group of lines only two testcrosses were not different (LSD, 0.05) than driest check hybrid DKC33-54 for grain moisture at harvest. The result showed that the SSS lines represent medium to late maturity (>83 RM) for ND. Lines derived from tropical Mexican hybrid DKB844 produced many hybrids which had 10-50 g kg^{-1} less grain moisture as compared to B73 derived hybrids (Figure 5). BSSS derived lines that were used as checks had shown some promise to low moisture. However, they still lack other important traits for selection under short-season environments. Lines derived from tropical Mexican hybrid DKB844 had a maturity similar to 87RM hybrid Pioneer 39D85. Therefore, they were adapted to this range of maturity as compared many of the lines derived from temperate US lines B73 and BSSS. The US lines used as checks may need one or more backcrosses to the adapted parent. Overall, the incorporation process was successful to generate lines with less than 90RM lines with one generation of backcross to adapted lines.

The plant height and ear height of checks was taller or within the range of testcross hybrids (Tables 1 and 2). There was a significant reduction in plant

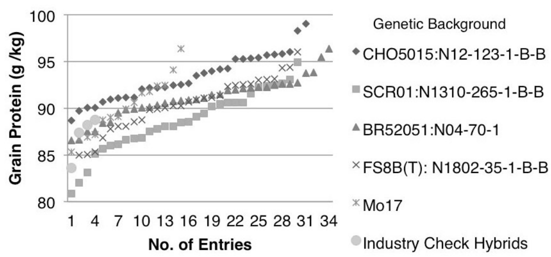

Figure 5 - Grain protein mean distribution of testcross maize hybrids representing different NSS genetic backgrounds [experimental mean was 91 g kg^{-1} and LSD (0.05) was 6 g kg^{-1}].

height in hybrids derived from exotic germplasms as compared to their initial stage of adaptation in ND short seasons. This showed the incorporation with adapted lines and visual selection in nursery was sufficient to decrease the height of exotic hybrids to the range of commercial hybrids. Successful development of relatively photoperiod insensitive inbreds from tropical germplasms was also reported by Holly and Goodman (1988). They found ear hight and days to flowering of exotics derived from 100% tropical within the range of commercial hybrids.

Adapted lines showed the ability to have similar or higher yield as compared to check hybrids and check temperate parents (Tables 1 and 2). There were 45 testcrosses out of 144 entries not different (LSD, 0.05) to the highest yielding entry among NSS lines. Only 19 testcrosses were statistically not different to the highest yielding entry among SSS lines. The yield advantage with tropical exotic incorporation was also reported by Holland and Goodman (1995). Most adapted CHO5015 and BR52051 derived lines showed high yield in testcross combination (Table 1). Some of the lines from tropical SCR01 and FS8B(T) derived hybrids also showed high yield as compared to many of the lines derived from the temperate parent Mo17 (Figure 6). BC derived line [(GEM 5 x ND 2000) x ND 2000)-1]-9 showed to be outstanding for high yield (7.2 Mg ha^{-1}) and low grain moisture (218 g kg^{-1}) in ND (Table 2). This line showed a new insight to breeding for earliness contrary to the common belief that earliness is combined with low yield in hybrids (Howbaker et al, 1997). Probably, a larger sample size demonstrated both traits could be found together in a desirable way as well. CUBA 117(GEM 3) derived from a tropical exotic breeding cross and one of its derived early versions [(GEM 3xND2000) xND2000-1]-81 produced testcrosses with intermediate (similar to 87RM Pioneer 39D85) grain moisture at harvest and high yield (Table 3). Hybrids with B73 genetic background showed high yield and high grain moisture while lines derived from BSSS had low yield.

Exotic derived BC lines with low grain moisture at harvest and comparable yield with industry checks can be an important genetic resource to develop the next generation of short-season diverse and unique maize hybrids while helping move maize north. The exotic germplasm incorporation was found to be unique to increase genetic diversity in temperate areas of US. The recovery of competitive inbred lines with acceptable grain moisture content at harvest derived from 100% tropical exotic germplasms was also reported in NC State (Hawbaker et al, 1997). Uhr and Goodman (1995) showed that among 190 lines derived from seven tropical commercial hybrids, 16 testcrosses were within the LSD (0.05) of the commercial checks for yield, standability, and grain mois-

Table 3 - Top 18 testcross means¶ among NSS† lines with low moisture and high yield across six ND environments in 2009 and 2010.

Entries	H_20 at Harvest	Grain Yield #	Stalk Lodge	Root Lodge §	Ear Height §	Plant Height §	Anthesis Date	Silk Date §
	(g kg^{-1})	(Mg ha^{-1})	(%)	(%)	(cm)	(cm)	D #	D #
TR3026xTR2040x[(GEM 5 x ND 2000) x ND 2000)-I]-212	253	7.2	0.4	1.6	84.9	206.6	67	69
TR3026xTR2040x[(GEM 5 x ND 2000) x ND 2000)-I]-9	218	7.2	1.1	0.0	90.2	207.7	65	67
TR3026xTR2040x[(GEM 5 x ND 2000) x ND 2000)-I]-97	253	7.0	1.0	0.7	95.0	212.4	66	68
TR3026xTR2040x[(GEM 5 x ND 2000) x ND 2000)-I]-219	241	7.0	0.5	0.0	87.7	208.6	66	67
TR3026xTR2040x[(GEM 22 x ND 2000) x ND 2000)-I]-51	245	6.9	0.8	1.8	98.3	216.0	66	69
TR3026xTR2040x[(GEM 5 x ND 2000) x ND 2000)-I]-54	242	6.8	1.6	0.0	100.0	223.1	66	69
TR3026xTR2040x[(GEM 5 x ND 2000) x ND 2000)-I]-33	232	6.8	1.1	0.0	93.5	212.8	67	69
TR3026xTR2040x[(GEM 4 x ND 2000) x ND 2000)-I]-25	252	6.8	1.3	0.2	90.6	212.5	68	67
TR3026xTR2040x[(GEM 22 x ND 2000) x ND 2000)-I]-39	250	6.8	0.3	2.0	96.0	208.1	66	68
TR3026xTR2040x[(GEM 5 x ND 2000) x ND 2000)-I]-24	218	6.8	1.4	0.8	92.5	212.5	66	67
TR3026xTR2040x[(Gem26 x ND2000)xND2000)-1]-44	220	6.6	0.7	0.5	87.4	197.5	65	67
TR3026xTR2040x[(GEM 22 x ND 2000) x ND 2000)-I]-67	247	6.6	0.4	0.8	90.6	210.4	66	67
TR3026xTR2040x[(GEM 4 x ND 2000) x ND 2000)-I]-47	244	6.5	2.7	4.2	88.9	210.6	66	67
TR3026xTR2040x[(Gem26 x ND2000)xND2000)-1]-108	246	6.5	1.4	0.1	90.5	205.6	65	67
TR3026xTR2040x[(GEM 22 x ND 2000) x ND 2000)-I]-45	230	6.5	1.9	0.5	95.9	214.1	65	67
TR3026xTR2040x[(GEM 5 x ND 2000) x ND 2000)-I]-19	237	6.5	0.0	0.5	83.2	206.4	65	66
TR3026xTR2040x[(GEM 22 x ND 2000) x ND 2000)-I]-98	243	6.5	2.0	0.1	92.7	209.9	65	66
TR3026xTR2040x[(Gem26 x ND2000)xND2000)-1]-35	250	6.5	0.6	0.5	86.3	200.8	65	67
NP2623CBLL x TR3030	273	7.9	0.0	0.2	112.6	227.6	70	70
Pioneer 39D85	251	7.5	0.0	0.0	102.6	216.9	66	68
DKC33-54	222	6.9	0.6	0.5	88.1	206.5	65	66
TR3127GT x TR3621CBLLRW	307	6.7	0.0	0.5	97.9	220.4	69	70
Exprimental Mean	258	6.2	1.1	0.9	90.7	207.9	66	68
$LSD_{0.05}$	29	1.4	2.7	4.7	5.7	8.3	2	2

†means adjusted for lattice effects.
‡Grain yield adjusted at 155 gm of water (H_2O) per kilograms of grain.
§Genotype x Environment interaction was not significant at 0.05 level of significance.
¶non stiff Stalk synthetic group.
#Days after planting.

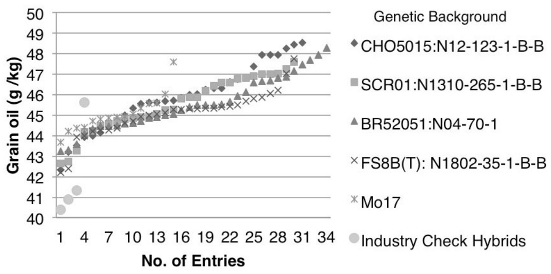

Figure 6 - Grain oil mean distribution of testcross maize hybrids representing different NSS genetic backgrounds [experimental mean was 45 g kg^{-1} and LSD (0.05) was 2 g kg^{-1}].

ture. Our results also showed that short-season hybrids with low grain moisture and comparable yield with commercial checks can be produced with exotic derived lines (Figure 6).

Grain quality adds value to short-season hybrids grown in challenging environmental conditions subject to rapid climate change (Carena et al, 2009). Exotic germplasm is a unique source for value added traits (Milton, 1970; Sing et al, 2001a; Pollak, 2003) and for the next generation of healthier hybrids. The exotic germplasm incorporation carried by the NDSU EarlyGEM program diversified the selection process carried within the NDSU maize breeding program by developing exotic lines with high quality traits. Accessions SCRO1 (GEM4) followed by BR52051 (GEM5), CHO5015 (GEM22), and CUBA117 (GEM3) derived BC lines produced several early testcrosses with grain oil and protein content higher than industry checks and BC line checks (Figure 6). The SCRO1 and CHO5015 derived exotic testcrosses had high grain oil content with medium maturity (Figure 6). CHO5015 accession contributed unique lines that produced testcrosses with high grain protein content (99 g kg^{-1} vs. 88 g kg^{-1}) compared to industry checks. CUBA117 derived lines had shown unique alleles for high protein content (102 g kg^{-1} vs. 91 g kg^{-1}) in testcrosses as compared to the industry check Pioneer 39D85. Exotic lines have donated unique exotic alleles for quality traits to produce higher quality <90RM lines. GEM accessions were also reported to have high protein and fat content than commercial hybrids in Iowa locations (Singh et al, 2001). DKB844 derived testcrosses showed high extractable starch content (643 g kg^{-1}) followed by BSSS and B73 derived testcrosses. These lines also produced testcrosses with high fermentable starch (HFC) and total starch content. High HFC and extractable starch observed from DKB844 derived testcross hybrids showed these new adapted lines can improve ethanol properties in future hybrids. The need for developing hybrids with a higher fermentable starch and extractable starch content for ethanol utilization was discussed by Bothast and Schlicher (2005). Unique genetic diversity was created for grain quality traits and can be another unique way to add value to short-season hybrids.

The US grain system is undergoing increased product differentiation and market segmentation (Elheri, 2007). These unique value added traits can fulfill the aspirations of farmers to diversify their products with specific traits and market demand.

The retention of exotic alleles during incorporation was high as showed by the high yield and high quality observed in both heterotic groups of lines with opposite heterotic group of testers as well as the visual characteristics of each line. Similar large distributions of means were observed across breeding populations (Figure 2 to Figure 9). All breeding crosses were unique, integrating diverse alleles to the lines and had potential to produce large range of lines for our breeding program. However, the probability of identifying unique alleles was higher across exotic breeding crosses than selecting within single BC populations. This result supports giving priority to sampling across rather than within populations in order to maximize utilization of genetic diversity (Holland and Goodman, 1995; Uhr and Goodman, 1995). Tartar et al (2003; 2004) showed that the higher genetic diversity observed in early generations can be maintained throughout the inbreeding period in inbred line development. The same trend has been observed in some of the advanced generation of exotic derived lines from the NDSU EarlyGEM program as they are among the top performing lines in multi-location hybrid trials.

The results differentiated the diverse $BC_1:S_1$ lines according to their merit for different traits. As a consequence, not only advanced lines have been identified but also nine new NDSU EarlyGEM breeding populations have been developed. A bulk-entry method has been utilized to develop the new exotic synthetic varieties with top lines for each trait. Reciprocal recurrent selection will follow to maximize their genetic improvement. These included the lines derived from accession BR52051 (<83RM and high grain protein), CHO5015 (early maturing high protein), SCR01 (High oil lines), CUBA117 (High protein), FS8B (High starch), and tropical hybrid DKB844 (High starch). In addition, three additional populations from Argentinia accessions have been created. Lines utilized in developing these new populations were included in the NDSU pedigree selection program for further early and late generation testing and inbreeding to develop early maturing hybrids with value added traits.

Conclusions

In ND, maize is being pushed north and west into marginal regions defined by short seasons, cold weather, and drought. The incorporation of unique exotic germplasm has shown to be a successful way to quickly adapt maize into these marginal areas, utilizing late tropical and temperate exotic germplasm and developing early maturing lines and populations with diverse alleles. The existing US germplasm (B73, Mo17, BSSS, and commercial checks) do not carry diverse alleles to break these environment margins.

The most recycled and genetically narrow germplasm needs extensive efforts of imrpovement and they do not seem to have the diversity needed to adapt to more northern and cooler environments. The useful genetic diversity generated by the NDSU EarlyGEM program is a new and unique source of diverse tropical and temperate alleles for yield and quality under abiotic stresses for short-season hybrids not present in the hybrids offered to farmers by the US industry (eg., lines continue to be recycled from B73, Mo17, and others with most DNA known sequences). Utilizing unique germplasms and environments can be a successful way to develop desirable traits in a cultivar and adapt crops to climate changes. The NDSU EarlyGEM program has generated a unique pool of short-season germplasm (<90RM). Even though the exotic incorporation of unique germplasm and the application of long-term ideas are not a popular practice across public and private sectors, the results of this continuous research show otherwise. The results showed the multi-stage cooperative effort from LAMP, GEM and EarlyGEM is a successful way to increase the genetic diversity of breeding programs and hybrids on farms. As a consequence, research emphasis in applied breeding has shown the importance of public breeding programs to evaluate and improve elite exotic germplasms for selection in more diverse environments while targeting needed traits across breeding methodologies.

Acknowledgements

The research was conducted with partial funding from USDA-GEM program and North Dakota Corn Utilization Council. We also wanted to acknowledge Monsanto Co. for providing grain quality screening machines and calibrations.

References

Briggs FN, Allard RW, 1953. The current status of the backcross method of plant breeding. Crop Sci 45: 131-138

Bothast RJ, Schlicher MA, 2005. Biotechnological processes for conversion of corn into ethanol. Appl Microbiol Biotechnol 67: 19-25

Buckler ES, Holland JB, Bradbury PJ, Acharya CB, Brown PJ, Browne C, Ersoz E, Flint-Garcia S, Garcia A, Glaubitz JC, Goodman MM, Harjes C, Guill K, Kroon DE, Larsson S, Lepak NK, Li H, Mitchell SE, Pressoir G, Peiffer JA, Oropeza Rosas M, Rocheford TR, Romay MC, Romero S, Salvo S, H Sanchez Villeda, da Silva HS, Sun Q, Tian F, Upadyayula N, Ware D, Yates H, Yu J, Zhang Z, Kresovich S, McMullen MD. 2009. The genetic architecture of maize flowering time. Science 325: 714-718

Carena MJ, Bergman G, Riveland N, Eriksmoen E, Halvorson M, 2009a. Breeding maize for higher yield and quality under drought stress. Maydica 54: 287-296

Carena MJ, Pollak L, Salhuana W, Denuc M, 2009b. Development of unique and novel lines for early-maturing hybrids: moving GEM germplasm northward and westward. Euphytica 170: 87-97

Carena MJ, Wanner DW, 2003. Registration of ND2000 inbred line of maize. Crop Sci 43: 1568-1569

Coles ND, McMullen MD, Balint-Kurti PJ, Pratt RC, Holland JB, 2010. Genetic control of photoperiod sensitivity in maize revealed by joint multiple population analysis. Genetics 184: 799-812.

Coles ND, Zila CT, Holland JB, 2011. Allelic effect variation at key photoperiod response quantitative trait loci in maize. Crop Sci 51: 1036-1049

CSSA, 2011 Position Statement on Crop Adaptation to Climate Change. Crop Science Society of America, Madison, WI

Dudley JW, 1982. Theory for transfer of alleles. Crop Sci 22: 631-637

Elbehri A, 2007. The Changing Face of the US Grain System: Differentiation and Identity preservation trends. United States Department of Agriculture Economic Research Service. Economic Research report number 35

Germplasm Enhancement of Maize Project, 2010. GEM Releases [online]. Available at http://www.public.iastate.edu/~usda-gem/OfficialDocuments/GEM_Germplasm_Releases.htm. (accessed May 4, 2011)

Goodman MM, 1990. Genetic and germplasms stocks worth conserving. Journal of Heredity 11-16

Goodman MM, 1999. Broadening the genetic diversity in maize breeding by use of exotic germplasm, pp. 139-148. In: The genetics and exploitation of heterosis in crops. Coors JG, Pandey S eds. ASA-CSSA-SSSA, Madison, WI

Goodman MM, 2005. Broadening the US maize germplasm base. Maydica 50: 203-214

Hallauer AR, 1978. Potential of exotic germplasm for maize improvement. In: Maize breeding and genetics. Walsen DB ed. John Wiley and Sons, New York

Halluer AR, Carena MJ, 2009. Maize breeding, pp. 3-98. In: Carena MJ ed. Handbook of plant breeding cereals. Springer, New York

Hallauer AR, Miranda Fo JB, Carena MJ, 2010. Quantitative genetics in maize breeding. 3rd ed, Springer, NewYork, NY

Holland JB, Goodman MM, 1995. Combining ability of tropical maize accessions with US germplasm. Crop Sci 35: 767-773

Holley RN, Goodman MM, 1988. Yield potential of tropical hybrid maize derivatives. Crop Sci 28: 213-218

MBS Genetics Handbook, 2008, 2010. Martin Bryton Seed Co, Ames, Iowa

Howbaker MS, Hili WH, Goodman MM, 1997. Application of recurrent selection for low grain moisture content at harvest in tropical maize. Crop Sci 37:1650-1655

Mikel MA, Dudley JW, 2006. Evolution of North American dent corn from public to proprietary germplasm. Crop Sci 46: 1193-1205

Milton DJ, 1970. Plant introduction of maize as a source of oil and unusual fatty acid composition. J Agr Food Chem 18: 1970

Pollak LM, 2003. The history and success of the public private project on Germplasm Enhancement of maize (GEM). Adv Agron 78: 45-87

Ransom J, Franzen D, Glogoza P, Hellevang K, Hofman V, McMullen M, Zollinger R, 2004. Basics of corn production in North Dakota. http://www.ag.ndsu.edu/pubs/plantsci/rowcrops/a834w.htm (assessed March 23, verified June 27, 2011). NDSU Extension Service, NDSU, Fargo, ND

Russell WA, 1972. Registration of B70 and B73 parental lines of maize. Crop Sci 12: 721

Sevilla R, Salhuana W, 1997. General description of the plan and execution of Latin American Maize Project (LAMP), pp.1-35. In: Salhuana W, Sevilla R, Eberhart SA eds. LAMP Final Report 1997

Simmonds NW, 1993. Introgression and Incorporation: Strategies for the use of crop genetic resources. Biological reviews 68: 539-562

Singh SK, Johnson LA, Pollak LM, Hurburgh CR, 2001. Compositional, physical and wet-milling properties of accessions used in Germplasm Enhancement of Maize project. Cereal Chem 78: 330-335

Tarter JA, Goodman MM, Holland JB, 2004. Recovery of exotic alleles in semiexotic maize inbreds derived from crosses between Latin American accessions and a temperate line. Theor Appl Genet 109: 609-617

Troyer AF, 1999. Background of US Hybrid corn. Crop Sci 39: 601-626

Troyer AF, 2009. Development of hybrid corn and the seed corn industry, pp. 87-114, In: Maize Handbook - Volume II: Genetics and Genomics. Hake JL ed, New York, NY

Yang J, Carena MJ, Uphaus J, 2010. AUDDC: A method to evaluate rate of dry down in maize. Crop Sci 50: 1-8

Zuber MS, 1973. Registration of 20 maize parental lines. Crop Sci 13: 779-780