Registration of Five Short-Season Stiff Stalk (SS) EarlyGEM Maize Germplasms

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Abstract
In 2000, the North Dakota (ND) maize (Zea mays L.) breeding project initiated the long-term EarlyGEM program to increase the genetic diversity of northern US maize hybrids. The goal was to expand Germplasm Enhancement Maize (GEM) efforts. NDEarlyGEM3 (Reg. No. GP-594, PI 675374), NDEarlyGEM10 (Reg. No. GP-595, PI 675377), NDEarlyGEM21a (Reg. No. GP-596, PI 675378), NDEarlyGEM21b (Reg. No. GP-597, PI 675379), and NDEarlyGEM21c (Reg. No. GP-598, PI 675380) are new maize populations carrying diverse genetics for short-season environments. These populations belong to the Stiff Stalk (SS) heterotic group as per their original GEM pedigree validated by combining ability data. A modified pedigree selection program including elite GEM and ND lines was utilized to move GEM germplasm northward and westward. Early- and late-generation hybrid trials, including EarlyGEM BC1-derived lines, were conducted from 2006 to 2010. The bulk-entry method allowed top progenies not only to be recombined to create new populations but also to initiate the development of new elite lines and hybrids. New EarlyGEM populations provide a unique option to expand the gene pool of breeding programs. Four new EarlyGEM lines (ND2014, ND2015, ND2016, and ND2035) derived from released NDEarlyGEM SS populations have recently been released to industry exclusively after being tested across 51 to 64 northern US environments. In this paper, however, only EarlyGEM populations are registered due to the fact that elite EarlyGEM inbred lines have intellectual property exclusive restrictions set up by the North Dakota State University Research Foundation. Adaptation and improvement programs carrying unique and diverse germplasm will be essential in the development of new cultivars tolerant to climate changes.

Methods
Introduction of GEM Materials
Wellhausen (1965), Goodman and Brown (1988), and Salhuana (1997) had in common their willingness to establish international and national coordinated efforts to evaluate and incorporate exotic germplasm. The Latin American Maize Project (LAMP) evaluated 12,113 accessions for multiple traits across 12 countries in Latin America (Sevilla and Salhuana, 1997), and 268 accessions were identified as elite germplasm sources (Pollak, 2003). The US Congress appropriated funds to continue breeding efforts with LAMP accessions. As a

Abbreviations: GEM, Germplasm Enhancement Maize; LAMP, Latin American Maize Project; ND, North Dakota; NDSU, North Dakota State University; RCBD, randomized complete block design; RM, relative maturity; SS, Stiff Stalk.
consequence, the GEM program was created with the intent to broaden the genetic base of the hybrids grown by US producers.

Original GEM efforts concentrated on areas in the central and eastern United States. Different sets of accessions were identified and assigned to seed companies to make crosses with elite inbred lines (Fig. 1, Step I). In some cases, these breeding crosses were then assigned to different seed companies (Fig. 1, Step II) to make additional crosses (Salhuana, 1997). Company names were coded but heterotic group information was preserved. The GEM program later initiated inbred line development with early generation testing to provide elite S₃ lines to network cooperators (Fig. 1, Steps III, IV, and V). These lines were still segregating to allow for further selection. In the late 1990s, NDSU was one of the participating institutions in the network of public and private cooperators that grew significantly over the years.

The plant materials introduced to North Dakota were S₃ lines the GEM program made available to network cooperators.

**Adaptation of Genetic Materials to Short Seasons**

The EarlyGEM program began leading the efforts to incorporate tropical and late temperate genetic materials in the northern United States and southern Manitoba 15 yr ago. To expand GEM efforts, the North Dakota maize breeding project initiated a long-term program to increase the genetic diversity of hybrids in the northern United States with the rapid incorporation of elite exotic germplasm. The goal was to move elite tropical and temperate maize germplasm northward to short-season drought and cold-prone environments. In 1999 to 2000, the first set of top GEM S₃ lines derived from breeding crosses

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**Fig. 1.** Long-term breeding process followed to develop NDEarlyGEM lines and populations. The chart shows how the original set of GEM lines was adapted; more sets followed a similar process over the years (modified from Hallauer et al., 2010). GEM, Germplasm Enhancement Maize; LAMP, Latin American Maize Project; NDSU, North Dakota State University; SS, Stiff Stalk.
was acquired from Ames, IA. A total of 152 S₁ GEM lines were selected based on top grain yield and agronomic data generated by the GEM program, paying special attention to potential adaptation to shorter seasons (Fig. 1, Step VI). Seed of each GEM selected line was planted, one row each, for observation in the 2001 Fargo maize breeding nursery. Emphasis was placed on earliness, agronomic potential, and unique characteristics and trait combinations not observed before (e.g., disease resistance, quality, among others). Plants of all lines were still segregating. An average selection intensity of 18.4% was utilized, and the 28 S₁ GEM lines with most adaptation potential to short-seasons were crossed to released and experimental ND lines (Fig. 1, Step VII). As the GEM program released new sets of S₁ lines, the EarlyGEM program incorporated more sets, making this process continuous over the years.

A modified pedigree selection program was initiated including elite late maturing GEM lines and short-season ND lines to move US Corn Belt GEM germplasm northward and westward. Crosses between ND and first set of GEM lines were conducted in the 2002 Fargo maize breeding nursery. ND inbred line ND2000 (PI 631394; Carena and Wanner, 2003) was used as recurrent parent in most cases to produce BC₁;S₁ source populations (Fig. 1, Step VIII) as it yielded the most successful early by late crosses. ND2000 is a yellow-dent maize inbred line released by the North Dakota Agricultural Experiment Station in 2002 due to its excellent female parent characteristics and its potential to produce 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. Molecular markers have assigned this line to the Stiff Stalk (SS) heterotic group, but this assignment has not been fully reliable when evaluating testcross with industry and public testers. This line has shown good combining ability across SS and non-SS industry and public testers.

The inspiration of the NDEarlyGEM long-term adaptation program was the backcross-breeding program initiated by Dr. Pinnell at University of Minnesota (Rinke and Sentz, 1962) that yielded successful Minnesota “A” lines (e.g., early versions of BI4). But in this case, late-maturing GEM S₀ parents were used as donor parents while short-season lines were used as recurrent ones. In addition, only one backcross generation was produced without screening of F₁ generations to reduce the time for exotic inbred line development (Carena et al., 2009a). While BC₁ generations were produced in the 2003 Fargo maize breeding nursery, visual selection was used to discard late-maturing individuals with agronomic deficiencies (e.g., poor stands, low seedlings vigor, lodging, insect and disease susceptibility, and height). A selection intensity of 32.1% was applied. Thus, only nine populations were kept to produce originally about 5000 BC₁;S₁ early generation lines in the 2004 Fargo maize breeding nursery (Fig. 1, Step IX) for inbred line development and early generation testing. Populations included SS donors CUBA117;SI520-388-1-B (internally coded as GEM3), CHIS775;SI911b-B-B (coded as GEM10), and AR16026;SI7-66-1-B (coded as GEM21). GEM21 was used in the development of three new populations carrying different groups of lines. BC₁;S₁ lines followed the NDSU pedigree selection process, including drought and cold tolerance screening under managed stress winter nursery environments (Carena et al., 2009b). Remaining seed of each line was left in cold storage for future testcrossing.

**Evaluation of Genetic Materials**

Overall, testcross trials were used to evaluate the potential of NDEarlyGEM early-generation lines in hybrid combinations to create the new populations. In addition, diallel and North Carolina II mating designs were used to evaluate the release potential of NDEarlyGEM populations per se and in hybrid combinations. These populations have also served as successful sources of new advanced generation lines and hybrids, some of them already released after extensive testing.

Selection of early-generation lines was conducted under managed stress winter nursery environments in New Zealand and Argentina. The surviving BC₁;S₁ lines derived from the first set of GEM S₁ lines acquired were included in the first EarlyGEM testcross nurseries in the 2005 Fargo maize breeding nursery (Fig. 1, Step X). Long-term cooperators from foundation seed companies provided elite testers representing the most current early BI4, Iodent, and unrelated heterotic groups for early- and late-generation testing at that time. Preliminary screening during 2006 and 2007 reduced progeny numbers even further (Fig. 1, Step XI). In addition, marginal short-season drought tolerance trials were conducted and western North Dakota experiments included side-by-side irrigated and dry land trials. In the 2008 Fargo maize breeding nursery, additional testcrossing followed. In this case, newer industry testers were planted within two ranges of BC₁;S₁ selected lines for manual pollination purposes instead of traditional tester isolation and detasseling. The tester served as the female and was crossed to the adjacent BC₁;S₁ lines. This allowed utilization of BC₁;S₁ selected lines as males to advance one generation of self-pollination at the same time as utilizing tassels to cross to industry testers. Sharma and Carena (2012) added BC₁;S₁ checks that included B73 and Iowa Stiff Stalk Synthetic (BSSS) as donors. The progeny produced from each female row was shelled in bulk and was the source for testing in multilocation trials across North Dakota environments. Yield trials across multiple US northern locations per year were arranged in experiments ranging from 5 × 5 to 14 × 14 partially balanced single lattice designs (Fig. 1, Step XI).

Based on all data, the bulk-entry method was utilized in the recombination sections of Fargo maize breeding nurseries to intermate top progenies and develop the new populations (Fig. 1, Step XI). Top progenies from multi-environment trials were planted in short rows along with bulks, permitting the breeding program to have a manageable number of rows as opposed to utilizing other mating designs for recombination. The bulk-entry method lowered the overall cost of recombination by reducing the number of rows and pollinations needed. Table 1 shows the number of progenies utilized as effective population size in NDEarlyGEM released populations.

The bulk-entry method identified new early-generation lines that followed additional pedigree selection for the development of new lines and hybrids. Therefore, recombination between top progenies (males) and bulks (females) allowed us not only to create new short-season exotic populations but also to initiate the development of new elite lines and hybrids during the same season (Fig. 1, Step XI). Newly developed NDEarlyGEM populations were grown in the population maintenance sections.
of Fargo maize breeding nursery to produce Syn 2 populations (second recombination). Balanced bios from Syn 1 (first recombination) populations were used for this purpose. Crosses within each pedigree were produced among plants, avoiding full-sib family production. Tassels were removed from plants used as males and females to reduce the number of overall pollinations needed and to maintain as much equal representation as possible from gametes. In other words, the development of new populations included a two-step process. First, top lines were included in the recombination nursery section and the bulk-entry method was utilized to create Syn 1 populations. Second, the newly created populations were included in the population maintenance nursery section for additional recombination and seed increases. As a consequence, Syn 2 populations were created.

Mating designs were produced and evaluated to determine the agronomic potential of the newly developed genetically broad-based populations per se. A 16-diallel mating design included NDEarlyGEM21 populations with NDSU tropical and temperate genetic materials improved by recurrent selection (Laude and Carena, 2014). In addition, two North Carolina II mating design were produced, one between SS EarlyGEM populations and NDSU non-SS adapted populations in the single-cross (across heterotic groups) and population maintenance sections of Fargo maize breeding nursery. Both populations per se and population hybrids were produced. To represent each population, sample sizes were over 100 individuals (e.g., over 200 plants) for seed production and consisted of up to 10 rows per population or population hybrid. For population hybrid production, pair crosses were utilized between populations of the opposite heterotic group. On the other hand, for seed production of populations per se, tassels were removed from plants used as males and females to reduce the number of overall pollinations needed and to maintain as much equal representation as possible from gametes.

A total of 19 agronomic and quality traits were screened in 27 North Dakota environments. Top-performing industry hybrids representing the 80 to 95 relative maturity (RM) range were included as experiment control checks across experiments. They represented the maturity range for central and southern North Dakota and were annual benchmarks for all traits evaluated (Laude and Carena, 2014). Therefore, checks and testers were not the same across years. The target population for all trials was 90,000 plants ha\(^{-1}\). Top NDEarlyGEM lines were compared to commercial checks. Different benchmarks and heritability and rank-summation indices were utilized not only for release purposes but also for creating new and elite short-season exotic populations with different emphases on traits, as in the case of NDEarlyGEM21.

Additional testcrosses with more industry testers were conducted for newer sets of S\(_2\) GEM BC, populations and advanced lines of released populations. Annually, new testers were included as recommended by industry cooperators. The purpose of both early- and late-generation testing was to discard thousands of genotypes utilizing different experiments and testers over years to develop the next generation of diverse lines and hybrids.

### Data Collection and Statistical Analyses

Plots were planted and harvested with equipment adapted for small experimental plots. Husbandry practices were those recommended for maximum yields at the respective locations. Data were collected for each plot at each location for grain yield (t ha\(^{-1}\), adjusted on a 15.5% grain moisture basis), grain moisture at harvest (%), test weight (kg L\(^{-1}\)), stand (m ha\(^{-1}\)), percentage root and stalk lodging (%), emergence (%), plant and ear height (cm), and grain quality traits. A subsample of 500 g of kernels was collected from every plot and used to measure the grain quality of all the genotypes. The near-infrared spectroscopy grain analyzer (OmegAnalyzer G) was used as a rapid and nondestructive measure of analyzing quality parameters in maize grains.

Individual analyses of variance (ANOVARs) were computed using SAS (SAS Institute, 1990) for traits within environments. Data were collected and imported to SAS (SAS Institute, 1990) for analyses. The ANOVAs for each location were performed using the PROC LATTICE procedure. Entries were considered as a fixed factor, whereas replicates and blocks were treated as random. The relative efficiency of the lattice design with the randomized complete block design (RCBD) was calculated for each trait. If the relative efficiency was higher than 105%, adjusted means by incomplete blocks were utilized. If the efficiency was lower than 105%, means were not adjusted. For high efficiency traits, the effective error was used as denominator in the F-test instead of the RCBD error mean square. The traits with homogeneous variance were considered for combined analysis. Each location × year combination was considered as an environment. Adjusted and unadjusted means from each trait were utilized. Expected mean squares were based on a mixed linear model that considered environments and replications as random effects and entries as fixed effects. Combined error mean squares (pooled error) were calculated by pooling the correspondent individual error mean squares weighed by their corresponding degrees of freedom. Mean comparisons were assessed by Fisher’s protected least significant difference (LSD) since it has been shown to be an adequate test for detection of differences (Carmer and Swanson, 1971).
Results and Discussion

Goodman and Brown (1988) and Hallauer and Carena (2009) summarized the prebreeding activities of exotic germplasm of various breeding programs. This research shows a long-term continuous effort to incorporate GEM germplasm into the northern United States, which provides industry the chance to access unique genes for various traits commercially. New populations have shown to be outstanding sources for grain yield, grain quality, cold and drought tolerance, and significant disease resistance in short-season environments (Sharma and Carena, 2012).

Significant (P ≤ 0.05) differences were detected among entries (Table 2). Extensive data from mating designs showed that the five new NDEarlyGEM populations were very competitive per se when compared to adapted populations and in specific hybrid combinations across 27 environments. Moreover, new populations have served as excellent sources of elite short-season inbred lines and hybrids. Top BC1-derived lines were advanced and extensively tested across 51 to 64 short-season environments, in cooperation with industry. As a consequence, five new EarlyGEM SS lines (ND2014, ND2015, ND2016, ND2035, and ND2037), derived from released populations, were recently developed and shared with industry exclusively.

NDEarlyGEM lines and populations can be used as parents in current and future breeding programs. Exotic incorporations allow development of lines capable of carrying traits that are difficult to combine in the same hybrid (e.g., fast dry down with high yield, abiotic stress tolerance, high quality, and disease resistance). Ranks of adjusted means for lattice effects combined over environments were used to select BC1-derived lines for specific grain quality traits, grain yield, and earliness. Most selected lines in hybrid combinations were at least earlier and higher yielding than or similar to 87 RM day hybrid checks Pioneer 39D85 (Sharma and Carena, 2012). Exotic derived lines clearly added not only genetic diversity but also high-yielding alleles to the NDSU maize breeding gene pool. Thirty testcrosses were not significantly different (P ≤ 0.05) than the driest early-maturing check DKC33-54 for days of silking. On average, results showed that SS lines represented intermediate maturity for North Dakota when compared to industry checks (75–85 RM).

Sharma and Carena (2012) showed 49 testcrosses not different than the top check Pioneer 39D85 for grain starch content. NDEarlyGEM3, BSSS, and B73 testcrosses showed high grain oil content as compared to top industry check NP2623CBLL × TR3030 (4.8 vs. 4.4%) for grain oil composition. Most of the lines derived from NDEarlyGEM3 had the highest grain oil content as compared to industry checks and check populations. NDEarlyGEM3- and BSSS-derived lines also produced testcrosses with highest grain protein content as compared to top check hybrid TR3127GT × TR3621CBLLRW for grain protein content (10.4 vs. 9.3%).

NDEarlyGEM10- and NDEarlyGEM21-derived testcrosses showed the highest value and potential for ethanol and wet milling industry for line hybrids (Sharma and Carena, 2012). Heterotic grouping evaluation of population hybrids confirmed NDEarlyGEM21 to be a unique and alternative SS group (Laude and Carena, 2015).

Characteristics

The properties of each germplasm registered are based on both the combining ability information of top BC1-derived progenies recombined to form the new populations and the performance of populations per se and in hybrid combinations.

Table 2. Average of six traits for NDEarlyGEM populations per se compared with adapted populations per se and commercial hybrids, evaluated at 27 western and eastern North Dakota locations for 4 yr.

<table>
<thead>
<tr>
<th>Entries</th>
<th>Grain yield (t ha⁻¹)</th>
<th>Grain test weight (kg L⁻¹)</th>
<th>Grain moisture (%)</th>
<th>Grain starch (%)</th>
<th>Grain protein (%)</th>
<th>Lodging stalk</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDEarlyGEM3</td>
<td>6.24</td>
<td>69.2</td>
<td>13.9</td>
<td>70.1</td>
<td>10.4</td>
<td>8.7</td>
</tr>
<tr>
<td>NDEarlyGEM10</td>
<td>6.18</td>
<td>69.0</td>
<td>13.5</td>
<td>71.3</td>
<td>9.5</td>
<td>9.1</td>
</tr>
<tr>
<td>NDEarlyGEM21a</td>
<td>5.82</td>
<td>69.4</td>
<td>13.0</td>
<td>71.2</td>
<td>9.3</td>
<td>9.7</td>
</tr>
<tr>
<td>NDEarlyGEM21b</td>
<td>5.52</td>
<td>70.6</td>
<td>12.7</td>
<td>72.1</td>
<td>9.2</td>
<td>10.8</td>
</tr>
<tr>
<td>NDEarlyGEM21c</td>
<td>5.66</td>
<td>70.0</td>
<td>12.1</td>
<td>71.6</td>
<td>9.1</td>
<td>11.1</td>
</tr>
<tr>
<td>Mean (5)</td>
<td>5.88</td>
<td>69.6</td>
<td>13.0</td>
<td>71.3</td>
<td>9.5</td>
<td>9.9</td>
</tr>
<tr>
<td>NDBS1011†</td>
<td>6.31</td>
<td>67.9</td>
<td>16.4</td>
<td>70.0</td>
<td>9.2</td>
<td>6.8</td>
</tr>
<tr>
<td>NDBS11(FR-M)C3†</td>
<td>6.58</td>
<td>65.8</td>
<td>15.1</td>
<td>70.2</td>
<td>9.4</td>
<td>10.7</td>
</tr>
<tr>
<td>NDBSK(HI-M)C3†</td>
<td>6.79</td>
<td>65.7</td>
<td>18.1</td>
<td>69.7</td>
<td>9.3</td>
<td>9.3</td>
</tr>
<tr>
<td>Mean (3)</td>
<td>6.56</td>
<td>66.5</td>
<td>16.5</td>
<td>70.0</td>
<td>9.3</td>
<td>8.9</td>
</tr>
<tr>
<td>Hybrids (4)</td>
<td>7.06</td>
<td>63.2</td>
<td>14.8</td>
<td>70.6</td>
<td>8.9</td>
<td>9.1</td>
</tr>
<tr>
<td>LSD (0.05)‡</td>
<td>1.26</td>
<td>2.91</td>
<td>2.57</td>
<td>0.8</td>
<td>0.7</td>
<td>4.6</td>
</tr>
</tbody>
</table>

† Carena et al. (2008).
‡ Comparisons among entries.
The NDSU maize breeding program coordinated the generation of data across up to 64 northern US environments. Public and private institutions participated in the extensive testing efforts to identify the top progenies used in the development of the new populations proposed for registration (Sharma and Carena, 2012). In addition, mating designs data across 27 environments validated the potential of releases per se (Table 2).

NDEarlyGEM3

NDEarlyGEM3 is a unique maize population created at NDSU for breeding programs developing short-season SS diverse inbred lines with emphasis in grain protein. Twenty BC₁-derived lines with top hybrid yield and quality performance with non-SS industry testers across ND environments were recombined to form this new germplasm (Table 1). Large sample sizes of exotic germplasm allowed combining above-average grain yield and agronomic traits with excellent grain quality properties. Nine out of the 20 testcrosses were statistically ($P \leq 0.05$) similar to the top commercial check for grain yield, and 33 testcrosses were different statistically different from the earliest flowering industry check hybrid, DKC 33-54 (Sharma and Carena, 2012). These testcrosses included a tropical exotic breeding cross, and now they represent medium maturity (approximately 85RM) for the northern United States with premium grain quality.

As population per se, NDEarlyGEM3 has shown above-average grain moisture at harvest, plant and ear height, grain protein and lysine content, and grain starch content when compared to adapted ND populations and checks across 27 environments (Table 2). The protein quality, however, was not as high as the genotypes being developed within the Early Quality Protein Maize (QPM) and Early Quality Protein Maize for feedstock (QPMF) programs (Sharma and Carena, 2012). In combination with North Dakota Lancaster (PI 675364) population, a synthetic made of eight elite inbred lines (ND290, ND291, Mo17, Oh43, B100, CG44, CG63, and A619), NDEarlyGEM3 gave the largest population hybrid yield and top ranking genotype across locations (10.3 t ha⁻¹ in dryland nonmarginal North Dakota environments). It also had good combining ability with NDBS1101 and 9.2 t ha⁻¹ with NDBS11(FS-M)C3 in dryland nonmarginal North Dakota environments. As population per se, NDEarlyGEM10 has shown above-average grain moisture at harvest, plant and ear height, and grain starch content when compared to adapted North Dakota populations and checks across environments (Table 2).

NDEarlyGEM10 is a source of lines and hybrids with intermediate maturity for short seasons (approximately 83RM), high yield, and highest extractable starch content (industry-generated data not shown) for the wet milling and ethanol processing industry. The incorporation of exotic alleles from this germplasm will be important for hybrids targeted at ethanol production and starch-derived products.

NDEarlyGEM21a

NDEarlyGEM21a is a maize population created at NDSU for breeding programs developing short-season SS diverse inbred lines with emphasis in abiotic stress tolerance (Carena et al., 2009b; Zeze and Carena, 2009; Sharma and Carena, 2016). NDEarlyGEM21a was developed by recombining 16 BC₁ elite lines with top hybrid yield performance with non-SS industry testers across North Dakota environments. Late-maturing temperate germplasm was brought from Argentina to the central US Corn Belt through the USDA-GEM program and then adapted to the northern United States through the EarlyGEM late × early backcross adaptation program. Lines used to develop NDEarlyGEM21a were 12 to 14 d earlier maturing than original versions and showed greater grain yield in hybrid combinations than top industry hybrids, especially under short-season drought western North Dakota environments. The short-season drought tolerance efforts in these genetic materials included neither transgenic traits nor transgenic short-season checks for drought tolerance, as they were not available from industry. This was complemented with a new breeding methodology for high-throughput phenotyping of drought tolerance (Sharma and Carena, 2016), which did not need to dig roots and destroy plots under testing. Drought-tolerant lines and hybrids were identified with stable ranks across irrigated and dryland side-by-side experiments. Testcrosses including NDEarlyGEM21a BC₁-derived lines have also shown excellent grain quality properties with above-average test weight and top variants for oil, protein, and starch composition.

NDEarlyGEM21a was the first population utilized to develop new short-season EarlyGEM inbred lines to increase the genetic diversity of US northern hybrids. This germplasm
has provided new lines with high combining ability effects for grain yield and drought tolerance. It is a good source of short-season genotypes for the northern United States with approximately 80RM.

**NDEarlyGEM21b**

NDEarlyGEM21b maize population was created at NDSU for breeding programs developing short-season SS diverse inbred lines with high test weight and starch content. Recombining nine BC\(_1\)-derived lines that had top hybrid yield performance with non-SS industry testers across North Dakota environments developed this new population. The emphasis was extractable starch, and this population ranked first for this characteristic across all genotypes (Sharma and Carena, 2012), including commercial checks, demonstrating its uniqueness. Late-maturing temperate germplasm was brought from Argentina to the central US Corn Belt through the USDA-GEM program and then adapted to the northern United States through the EarlyGEM program. This population has unique alleles for high extractable starch and starch content for ethanol utilization.

Lines used to develop NDEarlyGEM21b were 15 to 17 d earlier maturing than original GEM S\(_3\) versions. In addition to very good performance in grain yield and grain moisture at harvest, testcrosses including NDEarlyGEM21b-derived lines have also shown excellent grain quality properties with above-average test weight and starch composition.

NDEarlyGEM21b showed good specific combining ability with NDBS21(R-T)C9 (PI 675364) for grain yield and grain oil content. As population per se, NDEarlyGEM21b has shown above-average grain moisture at harvest, test weight, plant and ear height, and grain starch content when compared to adapted ND populations and checks across environments (Table 2). It is a good source of short-season genotypes for the northern United States with approximately 78RM. NDEarlyGEM21b has been used commercially in population hybrids with high starch demand for ethanol utilization in central North Dakota environments.

**NDEarlyGEM21c**

NDEarlyGEM21c maize population was created at NDSU for breeding programs developing short-season SS diverse inbred lines with fast dry down and grain starch content. This new germplasm was developed by recombining 18 BC\(_1\) elite lines with top hybrid performance with non-SS industry testers across North Dakota environments. Late maturing temperate germplasm was brought from Argentina to the central US Corn Belt through the USDA-GEM program and then adapted to the northern United States through the EarlyGEM program. NDEarlyGEM21c carries alleles for low grain moisture at harvest, high test-weight, and high starch content. NDEarlyGEM21c is especially characterized by its fast dry down after the utilization of the nondestructive NDSU breeding methodology created for the accurate and high throughput phenotyping of rate of dry down (Yang et al., 2010). Lines used to develop NDEarlyGEM21c were 18 to 20 d earlier maturing than original GEM S\(_3\) versions. NDEarlyGEM21c showed good specific combining ability with NDBS1011 (PI 650888) (Carena et al., 2008), showing excellent grain yield and test weight.

As population per se, NDEarlyGEM21c has shown excellent dry down properties when compared to adapted ND populations and checks across environments (Table 2). It has successfully been used not only for the development of new short-season lines and genetically diverse hybrids but also in commercial population hybrids. NDEarlyGEM21c is a very good source of high-yielding short-season genotypes for the northern United States with approximately 75RM.

**Conclusion**

NDEarlyGEM3, NDEarlyGEM10, NDEarlyGEM21a, NDEarlyGEM21b, and NDEarlyGEM21c are unique and genetically diverse germplasm sources of short-season inbred lines and hybrids not found in current genome sequences or mapping populations, often utilizing very small and low-diversity samples. They are alternative options if maize breeders have an interest in expanding the base of their current breeding programs.

This research encourages more utilization of exotic germplasm for the generation of future maize sustainable cultivars. Results of this research provide evidence of the support needed for long-term adaptation and improvement programs, especially in maize. This supports the vision of Wellhausen (1965), Goodman and Brown (1988), Salhuana (1997), Pollak (2003), Hallauer (1999), Hallauer et al. (2010), Hallauer and Carena (2013), and Hallauer and Carena (2014), among others, to continue strong coordinated efforts to evaluate and incorporate exotic germplasm. As a consequence, this will encourage the development of the next generation of US sustainable hybrids carrying diverse genetics for future climate challenges. The NDEarlyGEM program is the first continuous breeding effort devoted to short-season germplasm enhancement with incorporation of tropical and temperate GEM genetic materials for inbred line development. Adaptation and improvement programs carrying unique and diverse germplasm will be essential in the development of new cultivars tolerant to climate changes.

**Population Maintenance and Distribution**

NDEarlyGEM maize germplasms were multiplied in Fargo, ND, and New Zealand breeding nurseries within their population maintenance nursery sections. Balanced bulks of 100 to 500 kernels were used for this purpose. Crosses within each section were produced among plants avoiding full-sib family production. Also, tassels were removed from plants used as males and females to reduce the number of overall pollinations needed and to maintain as much equal representation as possible from gametes. Lots of 200 kernels will be available for distribution, and NDSU breeder at Fargo, ND, will coordinate multiplication of populations for the first 5 yr.

**Availability**

North Dakota State University has transferred ownership of short-season materials to the NDSU Research Foundation (NDRF) (Carena, 2013). Therefore, requests of NDEarlyGEM germplasms should be made directly to Dale Zetocha (dale.zetocha@ndsu.edu). Material Transfer, Inbred Research,
Population Research, and/or Commercialization Agreements will need to be signed before the breeder is authorized to send seed lots. Breeder seed of NDEarlyGEM germplasms will be maintained by the NDSU maize breeding program and will be distributed (200 kernels per request) from the corresponding author on approval by NDRF during the first 5 yr. Seed of this material has also been deposited in the National Plant Germplasm System (NPGS) and will be freely distributed through NPGS 5 yr from the date of publication.

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References