GAINS FROM SELECTION DURING THE DEVELOPMENT OF SEMIEXOTIC INBRED LINES FROM LATIN AMERICAN MAIZE ACCESSIONS[1]

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ABSTRACT - Tropical maize represents the most readily accessible source of genetic diversity within the species but is unadapted to temperate production environments and thus not utilized on a large scale. M.M. Goodman has conducted a program to incorporate tropical landrace germplasm into the U.S. Corn Belt Dent gene pool for more than 30 years. During that time, about 1300 Latin American maize accessions underwent seven different stages of selection and breeding, resulting in 25 semiexotic inbred lines with superior testcross performance. Selection stages included: 1) selection for photoperiod insensitivity in populations derived from crosses between accessions and temperate inbred line Mo44, 2) testcross selection of noninbred semiexotic populations and 3) selection during inbreeding generations. To test the effects of the different stages of selection on agronomic performance, we evaluated 25 selected semiexotic lines and their 12 progenitor tropical maize accessions, and 47 noninbred semiexotic families resulting from three cycles of recurrent phenotypic selection for photoperiod insensitivity in populations derived from the same 12 accessions (C3 families). Each experimental entry was testcrossed to LH132 x LH51 and testcrosses were evaluated in eight North Carolina environments. Significant agronomic improvement was observed for grain yield, grain moisture, flowering time, ear and plant heights, lodging resistance and smut (*Ustilago zeae* (Beckm.) Unger) resistance when comparing mean C3 family testcross performance to the mean original accession testcross performance, but no additional improvement was observed in the inbred test-crosses. This implies that most gains in testcross performance occurred during the early generations of phenotypic selection for general adaptation. Selection during later inbreeding generations may have resulted in improved inbred *per se* performance, but did not result in improved testcross performance.

KEY WORDS: Germplasm; Landrace; Exotic; Diversity.

INTRODUCTION

Sixty years of interbreeding elite lines of maize derived from a small number of open-pollinated cultivars of the Corn Belt Dent race has led to a reduction in the genetic diversity of commercial U.S. cultivars (TROYER, 1999). SMITH *et al.* (1999) found that pedigree diversity of Pioneer brand inbreds and hybrids was lower in the 1990s than any previous decade. As of 1996, exotic germplasm contributed less than 3% to the pedigrees of U.S. maize cultivars (GOODMAN, 1999). Tropical maize germplasm represents a rich, but underutilized, source of exotic germplasm for incorporation into the temperate maize gene pool.

Tropical maize germplasm likely contains unique alleles that would be useful to temperate breeding programs (CROSSA and GARDNER, 1987; HOLLAND and GOODMAN, 1995; UHR and GOODMAN, 1995; TARTER *et al.*, 2003). However, working with tropical maize in temperate environments is hindered by photoperiod sensitivity (BROWN, 1975) and linkages between favorable alleles and alleles contributing to maladaptation (BROWN, 1953, 1988). Unfavorable characteristics of tropical germplasm grown in temperate environments include higher ear placement, poor root and stalk lodging resistance, low grain yields, high grain moistures, and susceptibility to smut (*Ustilago maydis* (Beckm.) Unger).

M.M. Goodman has conducted a tropical maize germplasm incorporation program at North Carolina State University (NCSU) for over 30 years. The NC-SU program has similar aims as the Latin American

Maize Program (SALHUANA *et al.*, 1991) and the Germplasm Enhancement of Maize program (POLLAK and SALHUANA, 2001), but has been conducted independently of those projects. In Dr. Goodman's program, exotic maize accessions were initially evaluated as populations *per se* and culled for agronomic performance (GOODMAN, 1983; CASTILLO-GONZALEZ and GOODMAN, 1989). Selected accessions were crossed to temperate-adapted line Mo44 to produce segregating semiexotic populations. Three cycles of recurrent selections for photoperiod insensitivity were conducted within each semiexotic population to produce cycle 3 (C3) families with improved adaptation to temperate environments. C3 families were evaluated for combining ability with Corn Belt Dent testers (HOLLAND and GOODMAN, 1995). Pedigree selection during inbreeding was then conducted within selected semiexotic lines to develop inbred lines. Finally, semiexotic inbred lines were selected based on testcross performance to a Corn Belt Dent tester (TARTER *et al.*, 2003).

We hypothesized that genetic gains have occurred at each stage of this selection program. A direct test of this hypothesis was not possible with previously published data, however, since materials derived from different stages of the selection program were evaluated with different testers and in different environments. In order to test this hypothesis, we selected 25 semiexotic lines that produced high yielding, agronomically acceptable testcrosses in a previous study (TARTER *et al.*, 2003) and evaluated them in the same experiment as their original 12 parental accessions. In addition, we included the C3 families from the same 12 accessions, including the direct progenitor family for each semiexotic inbred line, as well as the unselected families from the same accessions. All materials were crossed to the same temperate tester, LH132 x LH51, and testcrosses were evaluated in replicated trials in eight North Carolina environments. The objective of this study was to determine how much gain in testcross performance was achieved by specific stages of selection in the NCSU tropical germplasm incorporation program.

MATERIALS AND METHODS

Latin American maize incorporation program at NCSU

The NCSU program began by screening about 1300 typical Latin American maize accessions for agronomic utility (lodging resistance and pollination success rate) across two years in a daylength-neutral nursery in southern Florida (Stage 1, Fig. 1)

(GOODMAN, 1983). This screening program was initiated circa 1971 and continued until 1980 (M.M. GOODMAN, pers. comm.). CASTILLO-GONZALEZ and GOODMAN (1989) then evaluated 394 accessions that met minimal culling levels for yield and agronomic performance in replicated experiments in short-day environments (Stage 2). The 40 most promising accessions were selected for photoperiod conversion using Mo44 as the donor parent of photoperiod insensitivity during stage three of selection. Mo44 was derived from a cross between Mo22 and Pioneer Mexican Synthetic 17. It was selected as the temperate parent because of its photoperiod insensitivity and because it is largely unrelated to either of the two major heterotic groups of maize, Reid Yellow Dent or Lancaster Sure Crop (GERDES and TRACY, 1993). The 40 semiexotic populations derived from crosses between accessions and Mo44 were subjected to three cycles of recurrent phenotypic selection for early flowering in long-day environments in North Carolina (Stage 4). HOLLAND and GOODMAN (1995) tested the resulting "photoperiod-converted" cycle 3 (C3) families in testcrosses to A632*Ht* x B73*Ht* and to Mo17*Ht* x Oh43E. Based on multi-environment testcross evaluations, 69 C3 families were selected (Stage 5) as sources for inbred line development. Pedigree selection among and within lines was conducted during two generations of full-sib mating followed by two generations of self-fertilization (Stage 6) (TARTER *et al.*, 2003). TARTER *et al.* (2003) evaluated 164 semiexotic inbred lines developed during Stage 6 in testcrosses to LH132 x LH51. Based on mean testcross performance across 8 environments, they selected 25 superior semiexotic inbred lines (Stage 7). Some of these semiexotic inbred lines developed from these superior semiexotic families exhibited significantly greater grain yield than Mo44 in testcross, indicating recovery of favorable exotic alleles (TARTER *et al.*, 2003).

Experimental materials

Semiexotic inbred lines and their progenitors were selected from among 164 semiexotic inbred lines developed from crosses between Mo44 and tropical accessions, as described by TARTER *et al.* (2003). Twenty-five semiexotic inbred lines were selected based on greater grain yield than the experimental check (Mo44 x (LH132 x LH51)), acceptable silk-tassel synchrony, acceptable lodging resistance, and availability of early generation seed (Stage 7). Experimental entries included selected semiexotic inbred lines, the 12 corresponding original accessions from which they were derived, and four C3 families developed from each of the 12 accessions. An exception to this was that seed was available for only three of the four C3 families from Tuxpeño VEN 598. The set of entries tested consisted of 25 semiexotic lines, 47 C3 families, and 12 accessions. The plants within each C3 family had an expected inbreeding coefficient of approximately $F = 0.17$ due to finite populations sizes used during recurrent phenotypic selections. The inbred lines were derived from plants in a generation which had an expected inbreeding coefficient of $F = 0.74$ and the lines had expected homogeneity approximately equivalent to $F_{4:5}$ lines. The plants within the lines had an expected inbreeding coefficient of $F = 0.87$.

Testcross yield trials

Each semiexotic line, C3 family, original accession, and inbred Mo44 was crossed to the hybrid tester LH132 x LH51 for testcross evaluations. LH132 x LH51 was chosen because of its close relationship to the historically important Corn Belt Dent cross, B73 x Mo17. The experiment consisted of 90 entries, including 84 exotic or semiexotic testcrosses, experimental check

FIGURE 1 - Breeding scheme for the development of semiexotic inbred lines from cross between Latin American maize accessions and temperate parent Mo44.

Season	Material	Selection Stage
Winters 1971 - 1980's Homestead, FL (Short day photoperiod)	1300 accessions visually evaluated ↓ 394 superior accessions selected	Stage 1[1]
1983-1985 2 locations **Homestead, FL & Weslaco, TX** (Short day photoperiod)	394 typical racial accessions *per se* evaluated in replicated yield trials ↓ 50-75 accessions with superior performance identified	Stage 2[2]
Winter 1985-86 Homestead, FL	40 Accessions x Mo44 ↓ *C0 Seed: 4 full-sib 50% Exotic 50% Mo44* *semi-exotic families per cross*	Stage 3
Summer 1986 Clayton, NC	160 C0 Full-sib families | Intermated earliest flowering plants within a population ↓ *C1 Seed*	Stage 4
Summer 1987 Clayton, NC	C1 families | Intermated earliest flowering plants within a population ↓ *C2 Seed*	
Summer 1988 Clayton, NC	C2 families | Intermated among earliest flowering plants within a population ↓ *C3 Seed: 50% Exotic 50% Mo44* Inbreeding coefficient of this generation ~ 0.17 At time of harvest, 4 ears per population were selected and shelled.	
Summer 1991 3 NC locations	4 C3 families per 38 populations x 2 Corn Belt Dent testers yield trials – Year 1	Stage 5[4]
Summer 1992 3 NC locations	4 C3 families per 40 populations x 2 Corn Belt Dent testers yield trials – Year 2	
Clayton, NC	69 C3 families | Sib-mated within a row ↓ *C4 Seed* Inbreeding coefficient of this generation ~ 0.36	Stage 6[3]
Summer 1993 Clayton, NC	C4 families | Sib-mated within a row ↓ *C5 Seed* Inbreeding coefficient of this generation ~ 0.47	

FIGURE 1 - Continued.

Season	Material	Selection Stage
Summer 1994 Clayton, NC	C5 families | Self-fertilized ↓ *C6 Seed* Inbreeding coefficient of this generation ~ 0.74	
Summer 1995 Clayton, NC	C6 families | Self-fertilized ↓ *C7 Seed* Inbreeding coefficient of this generation ~ 0.87	
Summer 1999 3 NC locations	164 C7 semiexotic inbred lines x Corn Belt Dent tester yield trials – Year 1	Stage 7[4]
Summer 2000 4 NC locations	164 C7 semiexotic inbred lines x Corn Belt Dent tester yield trials – Year 2	

[1] Stage 1: Selection for accessions with sufficient agronomic value were based on standability and percent successful pollinations (M.M. GOODMAN, pers. comm.).

[2] Stage 2: Selections for accessions with good breeding value were based on grain yield, grain moisture, acceptable silk-tassel interval, lodging resistance, and overall agronomic performance.

[3] Stage 6: Individual plant selection performed at every generation. Pollinated plants were selected for good plant vigor, good viability, early flowering time, and low ear placement. At time of harvest, both plant and ear were selected against ear and stalk rot susceptibility as well as leaf disease susceptibility.

[4] Stages 5 and 7: Family selection and inbred line selection based upon mean performance for all agronomic traits in replicated yield trials of the C3 families and C7 inbred lines, respectively. Additionally, availability of C3 family seed sources was also considered during Stage 7 selection.

Mo44 x (LH132 x LH51), and five commercial hybrid checks (Pioneer brand hybrids 3165 and 31B13; DeKalb brand hybrids 714 and 743; and LH132 x LH51). The experimental design within a location was a 9 x 10 lattice design with two replications. Experiments were grown at each of four locations in North Carolina in years 2001 and 2002: the Central Crops Research Station near Clayton, the Peanut Belt Research Station near Lewiston, the Tidewater Research Station near Plymouth, and the Sand Hills Research Station near Jackson Springs. Soils at the experiment sites are classified as Marlboro Loamy Sand (clayey, kaolinitic, thermic Typic Paleudult) at Clayton, Norfolk Sandy Loam (fine-loamy, siliceous, thermic Typic Kandiudult) at Lewiston, Portsmouth Fine Sandy Loam (fine-loamy over sandy or sandy-skeletal, mixed, thermic Typic Umbraquult) at Plymouth, and Candor Sand (sandy, siliceous, thermic Arenic Paleudult) at Jackson Springs.

At all locations, plots were two 4.86-m rows sown with 44 seeds, with a 1-m length alley at the end of each plot. In Plymouth, Clayton, and Jackson Springs, rows were spaced 0.97 m, resulting in a population density of approximately 57,000 plants ha^{-1} within the planted plot area. In Lewiston, inter-row spacing was 0.91 m, and population density was 60,000 plants ha^{-1}.

Stand, mean plant height (height from the ground to the terminal node), and mean ear height (height from the ground to node of topmost ear) were recorded for each plot at all eight environments. Days to anthesis and silking were recorded only at Clayton in both years. Anthesis date was recorded when at least 50% of the plants in the plot were shedding at least 50% of the available pollen. Silk emergence date was recorded when 50% of the plants in the plot were displaying visible silks. Counts of the number of plants with any visible sign of smut infection (*Ustilago zeae*) were taken at all locations in 2002. At maturity, counts were taken of root-lodged plants (leaning greater than 30° from vertical with intact stalks) and stalk-lodged plants (broken below the ear or plants with dropped ears) per plot in all environments. Lodging resistance was measured as the percent erect plants in each plot that were neither root nor stalked lodged. Plots were machine harvested, and grain yield and moisture content were recorded for each plot. Grain yields were adjusted to 155 g kg^{-1} moisture content.

Statistical analysis

Data from each of the eight environments were analyzed for spatial trends as described by BROWNIE *et al*. (1993) using Proc MIXED in SAS version 8.0 (SAS INSTITUTE, 2000). Entry (experimental or check hybrid) was considered a fixed factor, whereas all other factors (replication, block within replication, and environment) were treated as random. For each environment, the following models were tested: a standard lattice design, a row and column trend analysis including up to fourth order orthogonal polynomials for both rows and columns, and a correlated errors model (BROWNIE *et al*., 1993). Models were chosen to minimize

the Akaike Information Criterion value and include only spatial trend factors significant at the $\alpha = 0.05$ level. Percent stand was included as a covariate for grain yield only when significant. Least square means from the spatial trend analysis for each environment were then combined across environments for the overall analysis. Appropriate least significant differences (LSDs) were calculated for comparisons of the C3 families testcross mean averaged across all accessions to the mean of all accession testcrosses or the Mo44 testcross mean. T-tests were performed to test the differences between the selected C3 families mean (those that were direct progenitors of the 25 selected semiexotic inbreds) and the mean of the all of the C3 families averaged across accessions without weighting. Unweighted means were used so that each accession contributed equally to the average value without regard to the number of C3 families that were selected within each accession. This was done by computing the difference between the mean of the selected families and the mean of all C3 families within each of the 12 accessions. The 12 differences were then averaged and tested for significance using the variance of those values. Similar unweighted T-tests were also used to test the mean differences between the selected C3 families mean and the semiexotic inbred lines mean averaged across accessions.

RESULTS AND DISCUSSION

Accession testcrosses ranged from 4.1 (VEN 767) to 6.3 (BOV 585) T ha^{-1} (Table 1). Mean yields of C3 families grouped within accessions ranged from 6.3 (VEN 981) to 6.9 (BAI III) T ha^{-1} (Table 1). In every case, C3 family testcrosses were numerically greater than their progenitor accession testcross, and these differences were statistically significant for 11 of the 12 accessions (BOV 585 was the exception; Table 1). Averaging across all 12 accessions, C3 family testcross yields were 1.4 T ha^{-1} greater than C3 accession testcrosses (Table 2), and the difference within an accession was as great as 2.4 T ha^{-1} (VEN 767, Table 1).

In addition to grain yields, significant gains from selection at stage 4 were observed for all agronomic traits measured (Table 2). Significant reductions in grain moisture, days to anthesis, days to silk, ear height, plant height and smut incidence were observed when comparing C3 family testcrosses to accession testcrosses. Additionally, the percentage of erect plants at maturity increased significantly in C3 family testcrosses. Reductions in plant height and time to flowering are desirable because most tropical maize is too tall and late-flowering for temperate production environments. These average responses, the results of the recurrent selection program, (Stage 4) were also observed in the differences between individual accessions and their C3 family descendants (Table 1). Seven of twelve (58%) C3 families differed from their accession progenitors for days to anthesis; all twelve C3 families differed for silk emergence; all twelve families also differed for both ear and plant height; eight (67%) families differed for smut incidence, and three (25%) families differed for grain moisture.

Part of the gain in testcross performance observed in the C3 family testcrosses, can be attributed to alleles from Mo44, which represents half of the parentage of each C3 family, and which also yielded significantly more than the accessions in testcrosses (Tables 1 and 2). However, the average testcross yield across all C3 families included in this study was also significantly greater than the testcross yield of Mo44 (Table 2). As a specific example, the mean testcross yield of C3 families from BAI III was 1.1 T ha^{-1} greater than Mo44 x (LH132 x LH51) (Table 1). These results indicate that recurrent phenotypic selection within segregating semiexotic populations (Stage 4, Fig. 1) was regularly successful at producing superior transgressive segregants.

Although C3 family testcrosses had significantly greater grain yield than Mo44 x (LH132 x LH51), they also had significantly greater grain moisture, ear height, and plant height, and significantly lower percent erect plants than Mo44 x (LH132 x LH51) (Table 2). Although significant, these differences were quite small. This result, and the fact that the C3 families on average did not differ from Mo44 for days to anthesis or days to silk, suggest that the photoperiod conversion program (Stages 3 and 4) was effective.

The effects of Stage 5 selection (choosing best C3 families based on testcross performance) were estimated by comparing all C3 families to the subset of C3 families that were used to develop the semiexotic inbred lines. Averaged across accessions, no significant differences were observed for any trait between the selected and unselected C3 families Table 2). No significant differences for grain yield were observed within specific accessions, either (Table 1). The effects of selection in Stage 6 (pedigree selection among and within lines during inbreeding) were estimated by comparing the testcross means of semiexotic inbred lines to the selected C3 families from which they were derived. Again, no significant differences were observed between these two groups, indicating that no gains in testcross performance were achieved from selection during inbreeding (Table 2). In one instance (ECU 770), the inbred line testcrosses were significantly

TABLE 1 - *Means of exotic accessions and semiexotic experimental families and lines testcrossed to (LH132 x LH51), Mo44 x (LH132 x LH51), and commercial checks evaluated in eight North Carolina environments.*

Race	Accession	Generation	No. Entries	Grain yield	Grain moisture	Erect plants	Days to anthesis[1]	Days to silk[1]	Ear height	Plant height	Smut Incidence
				Mg ha^{-1}	g kg^{-1}	%	— d —		— m —		%
Canilla	VEN 981	Accession	1	4.8	191	92	77	80	1.2	2.3	14
		All C3 Families	4	6.3	185	95	74	73	1.0	2.0	1
		Selected C3 Families	2	6.5	186	95	74	73	1.0	2.0	1
		Semiexotic Inbreds	2	6.6	182	95	76	74	1.0	2.0	2
Tuxpeño	VEN 598	Accession	1	5.0	207	95	81	81	1.3	2.5	13
		All C3 Families	3	6.5	188	96	72	73	1.0	2.1	1
		Selected C3 Families	1	6.3	189	95	72	74	1.0	2.1	2
		Semiexotic Inbreds	1	6.8	187	99	77	73	0.9	1.9	1
Tuxpeño	VEN 767	Accession	1	4.1	199	91	80	83	1.3	2.4	8
		All C3 Families	4	6.5	185	93	73	73	1.0	2.1	1
		Selected C3 Families	1	6.8	187	94	73	74	1.0	2.1	1
		Semiexotic Inbreds	1	6.5	185	98	73	75	0.9	1.9	0
Puya	MAG 322	Accession	1	4.5	206	94	79	84	1.3	2.4	6
		All C3 Families	4	6.4	186	96	72	74	1.0	2.1	0
		Selected C3 Families	1	6.6	188	96	72	73	1.0	2.1	0
		Semiexotic Inbreds	2	6.8	180	97	73	72	0.9	2.0	0
Puya	SAN 349	Accession	1	4.8	193	94	76	78	1.2	2.3	5
		All C3 Families	4	6.6	185	95	73	73	1.0	2.1	0
		Selected C3 Families	1	6.5	181	95	72	73	1.0	2.1	0
		Semiexotic Inbreds	1	6.7	183	96	72	74	1.0	2.1	0
Cubano Amarillo Duro	ECU 653	Accession	1	5.6	181	94	72	78	1.2	2.2	1
		All C3 Families	4	6.5	178	96	72	72	1.0	2.0	1
		Selected C3 Families	1	6.5	176	96	72	72	1.0	2.0	1
		Semiexotic Inbreds	1	6.6	177	97	71	73	1.0	2.1	1
Cubano Amarillo Duro	ECU 770	Accession	1	4.4	190	97	79	82	1.3	2.3	9
		All C3 Families	4	6.3	186	96	73	74	1.0	2.0	1
		Selected C3 Families	1	6.3	183	95	74	74	1.0	2.0	0
		Semiexotic Inbreds	2	6.8	189	95	72	74	1.0	2.0	1
Cubano Amarillo Duro	ECU 904	Accession	1	4.4	192	95	75	79	1.3	2.4	4
		All C3 Families	4	6.5	186	97	73	72	1.0	2.1	0
		Selected C3 Families	1	6.5	186	98	74	72	1.0	2.0	1
		Semiexotic Inbreds	7	6.3	186	98	70	72	1.0	2.0	0
Cubano Dentado	BOV 585	Accession	1	6.3	191	86	74	77	1.2	2.4	4
		All C3 Families	4	6.7	185	93	72	72	1.0	2.1	1
		Selected C3 Families	2	6.7	182	94	72	72	1.0	2.0	1
		Semiexotic Inbreds	2	6.9	179	97	70	73	0.9	2.0	0
Perla	LIM 13	Accession	1	5.9	184	90	74	77	1.3	2.4	3
		All C3 Families	4	6.7	189	97	74	74	1.0	2.1	0
		Selected C3 Families	2	6.7	189	96	75	73	1.0	2.1	0
		Semiexotic Inbreds	2	6.9	191	96	72	73	1.0	2.1	1
Tusón	BAI III	Accession	1	5.9	184	94	77	75	1.2	2.3	3
		All C3 Families	4	6.9	179	96	72	72	1.0	2.1	1
		Selected C3 Families	2	7.1	178	97	72	71	1.0	2.1	1
		Semiexotic Inbreds	2	6.9	180	95	73	72	1.0	2.0	1

TABLE 1 - *Continued.*

Race	Accession	Generation	No. Entries	Grain yield	Grain moisture	Erect plants	Days to anthesis[1]	Days to silk[1]	Ear height	Plant height	Smut Incidence
				Mg ha^{-1}	g kg^{-1}	%	—— d ——		—— m ——		%
Tusón	CUB 57	Accession	1	5.5	184	91	74	78	1.2	2.2	1
		All C3 Families	4	6.3	185	97	73	73	1.0	2.0	0
		Selected C3 Families	1	6.6	190	97	72	73	1.0	2.1	0
		Semiexotic Inbreds	2	6.4	183	96	74	72	1.0	2.1	1
Mo44 x (LH 132 x LH 51)			1	6.0	173	97	73	72	0.9	1.9	3
Commercial Checks			5	7.8	190	97	73	73	1.0	2.1	0
LSD$_1$[2]				0.6	13	4	3	3	0.1	0.1	3
LSD$_2$[3]				0.5	11	3	3	2	0.1	0.1	3
LSD$_3$[4]				0.4	8	2	2	2	0.0	0.1	2
LSD$_4$[5]				0.3	6	2	1	1	0.0	0.0	2

[1] Days to anthesis and days to silk recorded at Clayton only.
[2] LSD$_1$ is appropriate to compare two single entries at α = 0.05. To compare a mean of n_1 entries to a mean of n_2 entries, multiply the LSD given by $\sqrt{(1/2)(1/n1 + 1/n2)}$
[3] LSD$_2$ is appropriate to compare the mean of one entry to the mean of two entries at α = 0.05.
[4] LSD$_3$ is appropriate to compare the mean of two entries to the mean of four entries at α = 0.05.
[5] LSD$_4$ is appropriate to compare the mean of four entries to the mean of four entries at α = 0.05.

TABLE 2 - *Means of and differences between generations of semiexotic families and inbred lines corresponding to the stages of selection. Data are from 12 accessions and eight North Carolina environments.*

Experimental Material	Grain yield	Grain moisture	Erect plants	Days to anthesis[1]	Days to silk[2]	Ear height	Plant height	Smut Incidence
	Mg ha^{-1}	g kg^{-1}	%	—— d ——		—— m ——		%
Accessions Mean	5.1	192	93	76	79	1.2	2.3	6
All C3 Families Mean	6.5	185	96	73	73	1.0	2.1	1
Selected C3 Families Mean	6.6	185	96	73	73	1.0	2.1	1
Semiexotic Inbreds Mean	6.7	184	97	72	73	1.0	2.0	1
Mo44	6.0	173	97	73	72	0.9	1.9	3

Differences between Generation

All C3 Families Mean and Accessions Mean over all accessions

1.4*	−7*	3*	−3*	−6*	−0.2*	−0.2*	−5*

All C3 Families Mean and Mo44 over all accessions

0.5*	12*	−1*	0	1*	0.1*	0.2	−2

Selected C3 Families Mean and All C3 Family Mean over all accessions

0.1	0	0	0	0	0.0	0.0	0

Semiexotic Inbred Lines Mean and Selected C3 Families Mean over all accessions

0.1	−1	1	0	0	0.0	−0.1	0

[s] Days to anthesis and silk were records at Clayton only.
* Significantly different at P = 0.05.

higher yielding than their ancestral selected C3 family testcross, but in other accessions (CUB 57, VEN 767), the inbred line testcrosses were numerically lower yielding than the corresponding selected C3 family testcrosses (Table 1).

The apparent lack of genetic gain from selection in Stage 6 may be due to several causes. First, it is possible that selection for inbred *per se* phenotypes is not significantly correlated with testcross yields. Strong selection pressure for multiple disease resistances was applied during inbred development (Stage 6), and these phenotypes may have no correlation to yield in environments where the disease severity is limited. Additionally, selection for early-flowering genotypes was applied during inbred line development, and early-flowering plants are often lower yielding than later-flowering genotypes, within the adapted range of flowering time. Second, breeding value for testcross yield may depend strongly on specific dominance and epistatic interactions with tester alleles, and these cannot be evaluated in inbreds *per se*. Also, the estimates of gain from selection in Stages 5 and 6 may be biased because we evaluated only those families and accessions from which the best semiexotic inbred lines were developed. For example, inbred selection was initiated in 69 C3 families, but the set of 164 semiexotic inbred lines tested by TARTER *et al.* (2003) were derived from only 36 of those families (representing 23 different accessions). Then selection at Stage 7 was applied to choose the best 25 semiexotic inbred line testcrosses from among the set of 164 semiexotic lines tested. This reduced the number of tested accessions to the current subset of 12 accessions. Since these were likely the best starting populations, the amount of gain observed from selection within these populations may be lower than in the overall starting population. Another reason that the gain from inbred line selection may have been limited was that only limited numbers of lines representing original accessions were evaluated at any one generation of inbreeding and selection. This limitation was imposed on the program by the relatively small amount of resources available to the program and the desire to sample more accessions with fewer lines per accession rather than fewer accessions with more lines per accession. This strategy was chosen because the genetic variation for testcross yield was greater among than within accessions (HOLLAND and GOODMAN, 1995). Finally, we observed significant differences between semiexotic inbred line testcross yields and yields of all C3 families in some accessions (e.g., MAG 322 and ECU 770, Table 1). In these cases, there were small increases in yield due to both Stage 5 and Stage 6 and 7 selection, which were not individually significant, but were cumulatively significant. Averaged across all accessions, there were trends toward improvement in all traits in inbred lines compared to all C3 families, although the differences were not significant (Table 2).

Since we expect that the photoperiod conversion program selected against tropical alleles at photoperiod response loci and linked loci, the potential for loss of large amounts of exotic donor parent germplasm was a concern, as it would defeat the purpose of the project (to diversify the genetic base of temperate maize breeding pools). However, TARTER *et al.* (2004) estimated the proportion of exotic germplasm in the semiexotic lines derived from this program and found that the semiexotic lines retained at least 31% tropical alleles on average. These results, taken along with those of HOLLAND and GOODMAN (1995) and TARTER *et al.* (2003) indicate that the program was successful both at improving the testcross yields of semiexotic populations and at maintaining broad genetic diversity among the best lines selected from this project.

CONCLUSIONS

The superior performance of the semiexotic inbred line testcrosses, compared to their parental tropical accessions and also to their temperate parent (Mo44) in testcross, supports our hypothesis that alleles from exotic maize germplasm can contribute to improved productivity in temperate environments, even when the exotic sources *per se* appear to be entirely agronomically unfit. The semiexotic inbred line testcrosses derived from this multi-stage, long-term selection program do not exhibit their tropical parents' photoperiod sensitivity, lodging susceptibility, poor grain yields or high grain moisture. These lines represent unique sources of germplasm that can contribute to both enhanced diversity and productivity of temperate maize gene pools. However, we were surprised to observe that selection during inbred line development resulted in little or no gain in yield or other agronomic traits in testcross. This may have been due to a lack of correlation between inbred phenotypes and testcross yields.

REFERENCES

BROWN W.L., 1953 A summary of maize breeding techniques. Trop. Agric. **30:** 1-12.

BROWN W.L., 1975 A broader germplasm base in corn and sorghum. pp. 81-89. *In*: Proc. 30th Annu. Corn and Sorghum Industry Res. Conf. Chicago, IL. 10-12 Dec. American Seed Trade Association, Washington, D.C.

BROWN W.L., 1988 Plant genetic resources: A view from the seed industry. pp. 218-230. *In:* Seeds and sovereignty. Duke Univ. Press, Durham, NC.

BROWNIE C., D.T. BOWMAN, J.W. BURTON, 1993 Estimating spatial variability in analysis of data from yield trials: A comparison of methods. Agron. J. **85:** 1244-1353.

CASTILLO-GONZALEZ F., M.M. GOODMAN, 1989 Agronomic evaluation of Latin American maize accessions. Crop Sci. **29:** 853-861.

CROSSA J., C.O. GARDNER, 1987 Introgression of an exotic germplasm for improving an adapted maize population. Crop Sci. **27:** 187-190.

GERDES J.T., W.F. TRACY, 1993 Pedigree diversity within the Lancaster Surecrop heterotic group of maize. Crop Sci. **33:** 334-337.

GOODMAN M.M., 1983 Racial diversity in maize. pp. 29-40. *In:* D.T. Gordon (Ed.), Proc. of the Int. Maize Virus Disease Colloq. and Workshop. Ohio State Univ., Ohio Agriculture Res. and Development Center, Wooster, OH.

GOODMAN M., 1999 Broadening the genetic diversity in maize breeding by use of exotic germplasm. pp. 139-148. *In:* J.G. Coors, S. Pandey (Eds.), Genetics and exploitation of heterosis in crops. American Society of Agronomy, Madison, WI.

HOLLAND J.B., M.M. GOODMAN, 1995 Combining ability of tropical maize accessions with U.S. germplasm. Crop Sci. **35:** 767-773.

POLLAK L.M., W. SALHUANA, 2001 The Germplasm Enhancement of Maize (GEM) project: Private and public sector collaboration. pp. 319-329. *In:* H.D. Cooper, C. Spillane, T. Hodgkin (Eds.), Broadening the Genetic Base of Crop Production. CABI Publishing, New York.

SALHUANA W., Q. JONES, R. SEVILLA, 1991 The Latin American Maize Project: Model for rescue and use of irreplaceable germplasm. Diversity **7:** 40-42.

SAS INSTITUTE, 2000 SAS user's guide, Version 8.0., SAS Institute, Cary, NC.

SMITH J.S.C., D.N. DUVICK, O.S. SMITH, A. GRUNST, S.J. WALL, 1999 Effect of hybrid breeding on genetic diversity in maize. pp. 119-126. *In:* J.G. Coors, S. Pandey (Eds.), Genetics and exploitation of heterosis in crops. ASA, Madison, WI.

TARTER J.A., M.M. GOODMAN, J.B. HOLLAND, 2003 Testcross performance of semiexotic inbred lines derived from Latin American maize accessions. Crop Sci. **43:** 2272-2278.

TARTER J.A., M.M. GOODMAN, J.B. HOLLAND, 2004 Recovery of exotic alleles in semiexotic maize inbreds derived from crosses between Latin American accessions and a temperate line. Theor. Appl. Genet. **109:** 606-617.

TROYER A.F., 1999 Background of U.S. hybrid corn. Crop Sci. **39:** 601-626.

UHR D.V., M.M. GOODMAN, 1995 Temperate maize inbreds derived from tropical germplasm. II. Inbred yield trials. Crop Sci. **35:** 785-790.