Theor Appl Genet (2003) 107:798–805
DOI 10.1007/s00122-003-1341-x

R. S. Lewis · M. M. Goodman

Incorporation of tropical maize germplasm into inbred lines derived from temperate × temperate-adapted tropical line crosses: agronomic and molecular assessment

Received: 1 October 2002 / Accepted: 2 May 2003 / Published online: 12 July 2003

Abstract Exotic maize (*Zea mays* L.) germplasm may allow for increased flexibility and greater long-term progress from selection if it can be incorporated at high rates into U.S. breeding programs. Crosses were made between a temperate line, NC262A, and each of eight different lines consisting of 100% temperate-adapted tropical germplasm. Pedigree selection was used to generate a set of 148 F_5S_2 lines that were evaluated in testcrosses with FR992/FR1064 in nine North Carolina environments. Several entries had grain yield, grain moisture content and standability that were comparable to three commercial checks. The best testcrosses outyielded the cross NC262A × FR992/FR1064 by 9.5 to 10.9%, suggesting that a significant amount of tropical germplasm was retained in these lines and that this germplasm combined well with the Stiff Stalk tester. Previous researchers had suggested that tropical alleles could be rapidly lost during inbreeding in populations derived from tropical × temperate bi-parental crosses, leading to the development of lines that possess significantly less than 50% tropical germplasm. F_5S_5 sub-lines corresponding to the 14 best testcrosses were genotyped at 47 to 49 polymorphic simple sequence repeat (SSR) loci across all ten chromosomes to estimate the amount of tropical germplasm that was retained. The estimated genetic contribution from the tropical parent ranged from 32 to 70%, with the average being 49%. Only two of the 14 lines deviated significantly from a 50%-tropical/50%-temperate ratio, suggesting limited overall selection against germplasm from the tropical parents. These experiments collectively demonstrated that tropical maize germplasm can be incorporated at high rates into a temperate line via pedigree breeding methods in order to derive new inbred lines with acceptable agronomic performance.

Keywords *Zea mays* L. · Corn · Genetic diversity · Exotic germplasm · Simple sequence repeat

Communicated by D. Hoisington

R. S. Lewis (✉) · M. M. Goodman
Box 7620, Department of Crop Science, North Carolina State University, Raleigh, NC 27695, USA
e-mail: ramsey_lewis@ncsu.edu
Tel.: 919-513-4802
Fax: 919-515-7959

Introduction

The germplasm base on which most U.S. breeding is founded is considered to be extremely narrow (Goodman and Carson 1999). Most U.S. maize hybrids are almost entirely derived from a few open-pollinated varieties that descended from a mix of only two of 130 to 250 known races (Goodman and Brown 1988; Troyer 1999). Commercial U.S. breeders currently rely heavily on backcrossing and recycling of elite, often closely related, lines for developing improved hybrids (Troyer 2001). These 'advanced-cycle pedigree' approaches make great theoretical sense for achieving short-term genetic gains (Bailey and Comstock 1976; Bailey 1977; Dudley 1982, 1984), and U.S. maize yields continue to increase steadily at a rate of around 125.4 kg ha^{-1} per year (Duvick 1992; Troyer 1999). They can, however, result in a loss of genetic diversity among elite lines within a heterotic group (Lu and Bernardo 2001). There is strong incentive to continue use of these proven practices, but long-term progress and flexibility might be greater if germplasm pools were broadened through incorporation of new genetic variation (Hallauer 1978; Stuber 1978; Geadelmann 1984; Goodman 1985).

Some hybrids between 100% tropical lines and elite U.S. testers have been found to be competitive in yielding ability with commercial hybrids (Holley and Goodman 1988b; Godshalk and Kauffmann 1995; Uhr and Goodman 1995; Goodman et al. 2000). Resistance to several diseases has also been noted in tropical materials (Holland et al. 1998; Holley and Goodman 1988a, 1989; Uhr 1991; Tallury and Goodman 1999). Despite the obvious presence of favorable genes in all-tropical germplasm, its use in U.S. commercial hybrids increased only from 0.1% in

1983 to 0.3% in 1996 (Goodman and Carson 1999). Testcrosses involving all-tropical lines are often plagued by excessive lodging, late maturity, high grain moisture and barrenness under high planting densities (Holley and Goodman 1988b; Uhr and Goodman 1995). All-tropical inbred lines *per se* often exhibit problems such as poor cold tolerance, weak roots and stalks, poor tassel-silk synchronization, and susceptibility to smut and aphids (Goodman 1985, 1992).

Derivation of lines from crosses between all-tropical and U.S. temperate materials is one logical approach to remove many of the deficiencies contributed by the tropical parents. Private breeders would probably be more inclined to use lines with small amounts of tropical germplasm, because these would be most likely to satisfy short-term goals of generating products immediately suitable for commercialization. Lines consisting of 50%-tropical/50%-temperate materials, however, offer better long-range potential for broadening the U.S. maize germplasm base.

The description '50%-tropical/50%-temperate' is used in a rather loose sense above. In the absence of selection, randomly derived inbred lines from tropical × temperate line crosses would be expected to receive one-half of their nuclear genetic information from each parent. Depending on selection criteria, the tropical genetic contribution may actually be small due to selection against chromosome segments of the tropical parental genome during the inbreeding process. Previous researchers have suggested that extreme linkage disequilibrium coupled with intense selection could result in rapid loss of useful exotic genes in exotic × adapted populations unless extreme care is used in retaining plants showing exotic influence (Lonnquist 1974; Geadelmann 1984; Holley and Goodman 1988b). St. Martin (1982), however, indicated that selection of an inbred line with a 70% or greater genetic contribution from one parent would be very unlikely, even with intense selection pressure. Reductions in recombination between chromosomes from germplasm pools of diverse origin might also affect incorporation efforts (Lonnquist 1974).

The ultimate goal of 'incorporation' is the development of lines that are superior to parental lines and that contain high proportions of alleles not previously present in the immediate germplasm pool (Simmonds 1993). The first objective of this work was to determine if superior lines could be derived from crosses between 100% temperate-adapted tropical lines and a temperate line, NC262A, using conventional ear-to-row pedigree-selection procedures. A multi-stage testing approach was used to identify a set of the best-performing lines. A second objective of this work was to use simple sequence repeat (SSR) markers to estimate the tropical genetic contribution that remained in this superior group of inbred lines after inbreeding coupled with selection. Such data have implications for persons working to incorporate tropical germplasm into the temperate germplasm base. For example, the Germplasm Enhancement for Maize (GEM) project is a public/private collaborative effort that involves the generation of lines from crosses between elite Corn Belt lines and better Latin American maize accessions (Salhuana et al. 1994).

Materials and methods

Genetic material

Crosses were made between NC262A and each of the following 100% tropical lines (S_7 or S_8) in the summer of 1990: 7846-1, 7848-1, 7950-1, 7956-1, 7967-1, 7969-1, 7995-1 and 8020-1. These 100% tropical lines were derived using two cycles of pedigree breeding to adapt germplasm from lowland tropical hybrids to the United States (Table 1) (Goodman 1985; Holley and Goodman 1988b; Moreno-Mendoza 1989; Goodman et al. 1990). NC262A is a short inbred line with very low ear height and moderate resistance to gray leaf spot (*Cercospora zeae-maydis* Tehon and Daniels), anthracnose [*Colletotrichum graminicola* (Ces.) G.W. Wils.] and southern corn leaf blight [*Bipolaris maydis* (Nisikado) Shoemaker]. An estimated 112 days are required for this line to reach physiological maturity in Raleigh, North Carolina.

Table 1 Pedigrees and racial backgrounds of parental inbred lines

Line	Pedigree	Racial background
7846-1	105/H5//155[a]	Tuxpeño, ETO, Cuban Flint, Azteca, Tuxpan Yellow Dent
7848-1	105/H5//155	Tuxpeño, ETO, Cuban Flint, Azteca, Tuxpan Yellow Dent
7950-1	105/H5//H101	Tuxpeño, ETO, Cuban Flint, Tusón
7956-1	105/H5//H101	Tuxpeño, ETO, Cuban Flint, Tusón
7967-1	105/H5//H101	Tuxpeño, ETO, Cuban Flint, Tusón
7969-1	105/H5//H101	Tuxpeño, ETO, Cuban Flint, Tusón
7995-1	105//306/H5	Tuxpeño, ETO, Cuban Flint, Chandelle, Tusón, Coastal Trop. Flint
8020-1	105/H5//155/505	Tuxpeño, ETO, Cuban Flint, *A*zteca, Tuxpan Yellow Dent, Cateto
NC262A	McNair 14/McNair 16[b]	Corn Belt Dent, Southern Dent

[a] The following all represent tropical hybrids: 155 = Agroceres 155 (Brazil); 504 = Agroceres 504 (Brazil); 105 = Pioneer Brand X105A (Jamaica); 306 = Pioneer Brand X306B (Jamaica); H5 = H-5 (CNTA, El Salvador); H101 = H-101 (CNTA, El Salvador)

[b] McNair 14 and McNair 16 were derived from Coker 811A × C103[4]. Coker 811A was a modified double-cross of (C1 × C7) × [(C3R × C8R) × C4]. C1, C7, C3R and C4 were from Doufitts Prolific, Lathams Double, Biggs Two-Ear and Florida White, respectively. C8R was from a cross of lines from Florida White and Huffman. C103 is a first-generation inbred line of Lancaster origin

Ear-to-row selection was practiced within each pedigree in summer and winter nurseries in North Carolina and Florida, respectively. Five generations of plant-to-plant sib-mating within rows were followed by two generations of selfing. Plot sizes ranged from 12 to 25 plants, and 0 to 8 ears plot^{-1} were harvested each generation and planted ear-to-row the following generation. Among and within row selection during inbreeding emphasized early flowering, tassel-silk synchronization, low plant height, general ear quality (determined via a subjective visual rating), low ear moisture, lodging resistance and freedom from disease. Plots grown in North Carolina were inoculated with several pathogens including Races 1 and 2 of northern corn leaf blight [*Setosphaeria turcica* Luttrell (K.J. Leonard and E.G. Suggs)], southern corn leaf blight, gray leaf spot and anthracnose stalk rot. Those pedigrees that responded well to nursery selection were maintained, while those that did not were eliminated. In 1996, 148 F_5S_2 lines[1] were crossed with the single-cross tester FR992/FR1064 [representative of the Stiff Stalk Synthetic (Reid) heterotic group]. Lines were advanced for three more generations using ear-to-row selfing based on testcross yield-trial data.

Yield trials

Yield trials were conducted in 1997 through 1999 at three North Carolina locations: Clayton, Plymouth and Lewiston. In 1997, one experiment was also grown at Jackson Springs. Plots included two 4.86-meter rows with a 1-m pathway at the end of each plot. Between-row spacing was 0.97 m at Clayton, Plymouth and Jackson Springs, and 0.91 m at Lewiston. Plots were overplanted with 44 seeds plot^{-1} for a target plant density of approximately 43,000 plants ha^{-1} in Clayton, Plymouth and Jackson Springs, and 45,000 plants ha^{-1} in Lewiston. Data collected on all yield-trial plots included grain yield (t ha^{-1}), percentage grain moisture content at harvest, percentage lodging, plant height (cm from the base of a single representative plant to the tip of tassel) and ear height (cm from the base of single representative plant to the uppermost ear node). Days to midsilk and 50% pollen shed were observed only at the Clayton location.

Multi-stage testing was used to initially evaluate testcrosses of all lines and to subsequently identify a set of the best-performing materials. The set of 148 testcrosses was separated into two experiments to facilitate testing in 1997. Experiment #1 included 87 testcrosses of lines with the following pedigrees: NC262A/7950-1, NC262A/7956-1, NC262A/7967-1, NC262A/7969-1 and NC262A/7995-1. Experiment #2 included 61 testcrosses involving lines with the following pedigrees: NC262A/7846-1, NC262A/7848-1 and NC262A/8020-1. The experimental design for both experiments for the first year of testing was a randomized complete block with two replications. Commercial check hybrids Pioneer Brand 3165, DeKalb Hybrid 689 and LH132 × LH51 were included as checks in each experiment.

Based on cumulative testcross-performance data, the better testcrosses were selected for additional testing and combined into a single experiment. The main criteria for elimination were poor yield, high moisture or severe lodging. The 1998 experiment included 32 testcrosses. The experimental design was a 6 × 6 × 3 lattice with Pioneer Brand 3165, DeKalb Hybrid 689, DeKalb Hybrid 714 and LH132 × LH51 serving as checks. Based on cumulative performance during 1997 and 1998, 14 testcrosses were selected for a 3rd year of evaluation. The experimental design in the final year was a 4 × 5 × 3 lattice with Pioneer Brand 3165, Pioneer Brand 32K61, DeKalb Hybrid 689, DeKalb Hybrid 714, LH132 × LH51, and the three-way cross NC262A × FR992/FR1064 included as checks.

[1] Throughout this report, 'F_i' refers to the number of generations of sib-mating conducted after the initial hybridization. 'S_i' refers to the number of generations of self-pollination

Data analysis

Yield-trial data were subjected to analysis of variance procedures. Each location × year combination was considered as a single environment. Environments and entries were considered as random and fixed effects, respectively. Data from individual environments were first analyzed separately using methods appropriate for each experimental design (Cochran and Cox 1957). For experiments conducted using lattice designs, means adjusted for lattice effects were generated using the LSMEANS statement in PROC MIXED of SAS (Littell et al. 1996).

Combined analyses were conducted in two ways. For analysis of data from a single year, the combined analysis was conducted as described by McIntosh (1983). For the combined analysis over years, analysis of variance was performed on entry means from each environment. Only those entries grown in all environments were considered. The pooled error served as the entry × environment term in the analysis of variance. The entry × environment mean square was used as the error term for calculation of LSDs.

SSR experiment

F_5S_5 seed from single, self-pollinated ears of superior sublines corresponding to the 14 best testcross entries was collected. Extraction of DNA from these lines, NC262A, and each of the 100% tropical parents was performed according to Riede and Anderson (1996). Source material for each line was a bulk of 10–14 plants. A set of 159 SSR primer pairs (Research Genetics, Huntsville, ALA) amplifying loci on all 20 chromosome arms was screened for their ability to reveal polymorphisms between NC262A and the all-tropical parents. Polymerase chain reaction (PCR) conditions, electrophoretic separation of PCR reaction products and gel scoring were according to Senior et al. (1996). Those primer pairs found to produce readily interpretable polymorphic bands between the parents of each tropical × temperate derivative were screened against the selected F_5S_5 lines to estimate the genetic contribution from each parent.

A non-parametric procedure recommended by Bernardo et al. (1997) was applied to the marker data to determine if the number of SSR loci contributed from parent to progeny deviated significantly from that expected due to Mendelian inheritance. Only homozygous SSR loci were included in the analysis. The parental contribution of NC262A to a derived line was designated as p (after Bernardo et al. 1997). Ninety five percent confidence intervals (CIs) for estimates of p were obtained using a bootstrap re-sampling procedure (Bernardo et al. 1997). Expected and estimated temperate-parent genetic contributions were declared significantly different when the expected parental contribution was outside of the CI boundaries.

Results

Yield trials

Agronomic data for each of the 148 F_5S_2 testcrosses are not shown. The number of entries tested per pedigree during the first year is indicative of the pedigrees' response to nursery selection. Those pedigrees that responded poorly to selection had fewer entries. Better Experiment #1 and Experiment #2 pedigrees in terms of combined response to nursery selection and testcross yielding ability were those involving 7846-1 and 7995-1 as parents (Tables 2 and 3, respectively). This is not surprising because these two lines were identified as two of the best out of a set of second-cycle, all-tropical lines evaluated by Moreno-Mendoza (1989) in combination

Table 2 Agronomic trait means for Experiment #1 pedigrees (entries combined) evaluated in three North Carolina environments

Entry[a]	Yield (t ha^{-1})	Moisture (%)	Lodging (%)	Ear height (cm)	Plant height (cm)	Days to silk[b]	ASI[c] (days)
NC262A × 7950-1 F_5S_2 × T (15)	6.65*	16.4*	26.9*	87.6	255.8	79.5	0.5
NC262A × 7956-1 F_5S_2 × T (1)	6.31*	15.6	26.7	90.8	254.2	76.0*	0.5
NC262A × 7967-1 F_5S_2 × T (1)	6.90	17.7*	23.0	65.2*	277.5	80.0	1.0
NC262A × 7969-1 F_5S_2 × T (1)	6.50*	16.6	28.8	85.0	251.7	77.5*	0.0
NC262A × 7995-1 F_5S_2 × T (69)	6.73*	16.3	18.1	86.4	254.3	78.8	0.5
DeKalb Hybrid 689	7.53	16.6	13.3	105.8	258.3	82.0	0.0
LH132 × LH51	7.51	15.7	15.8	95.0	263.3	81.5	0.5
Pioneer Brand 3165	7.20	18.2*	20.8	105.8	279.2	85.0	0.0

*Indicates that the absolute difference between the pedigree or check mean and the mean for hybrid LH132 × LH51 exceeded the LSD ($P = 0.05$) calculated for making such comparisons according to Gomez and Gomez (1984)
[a] T = FR992/FR1064. The number of entries per pedigree is shown in parenthesis
[b] Flowering data collected at Clayton only
[c] ASI = anthesis-silking interval

Table 3 Agronomic trait means for Experiment #2 pedigrees (entries combined) evaluated in three North Carolina environments

Entry[a]	Yield (t ha^{-1})	Moisture (%)	Lodging (%)	Ear height (cm)	Plant height (cm)	Days to silk[b]	ASI[c] (days)
NC262A × 7846-1 F_5S_2 × T (20)	7.3	16.2	11.7	82.4	240.3	81.2	0.7
NC262A × 7848-1 F_5S_2 × T (22)	7.0	16.4	13.7	80.5	236.6	79.8	0.7
NC262A × 8021-1 F_5S_2 × T (19)	6.9	16.4	14.2	82.1	238.9	80.7	0.8
DeKalb Hybrid 689	7.3	16.2	11.7	82.4	240.3	81.2	0.7
LH132 × LH51	7.0	16.4	13.7	80.5	236.6	79.8	0.7
Pioneer Brand 3165	6.9	16.4	14.2	82.1	238.9	80.7	0.8

*Indicates that the absolute difference between the pedigree or check mean and the mean for hybrid LH132 × LH51 exceeded the LSD ($P = 0.05$) calculated for making such comparisons according to Gomez and Gomez (1984)
[a] T = FR992/FR1064. The number of entries per pedigree is shown in parenthesis
[b] Flowering data collected at Clayton only
[c] ASI = anthesis-silking interval

with two diverse testers. Sublines of these two parents have been released by the North Carolina State University as NC298 and NC300, respectively (Goodman et al. 2000).

Based on cumulative testcross performance, 14 testcrosses were evaluated for 3 years (nine environments). Only entries with 7995-1 or 7846-1 in their pedigrees remained for the 3rd year of testing because of nursery selection and culling due to poor yield-trial performance. Only one Experiment #1 testcross that was evaluated for 3 years had a grain yield that was significantly lower ($P = 0.05$) than that for the lowest-yielding commercial hybrid (Table 4). No experimental entry out-yielded this check, however. All selected testcrosses had grain moisture content at harvest that was within the range for the commercial checks. All experimental entries had less lodging (some significantly less) than Pioneer Brand 3165 and DeKalb Hybrid 689. Ear height for each selected testcross was significantly lower than the ear height for each commercial hybrid. All testcrosses also had plant heights that were lower (some significantly lower) than the mean plant height for the shortest commercial hybrid. Four of the seven selected Experiment #1 testcrosses reached midsilk significantly before the earliest flowering check hybrid, LH132 × LH51. None flowered significantly later than this check.

After 3 years of testing, no selected Experiment #2 testcross had a grain yield that was significantly different ($P = 0.05$) from that for the highest yielding commercial hybrid, Pioneer Brand 3165 (Table 5). Testcrosses of lines 1184-1 and 1191-3 with FR992/FR1064 out-yielded this check, although not significantly. Grain moisture for all selected Experiment #2 entries was within the range of grain moisture for the commercial hybrids. No testcross had significantly more lodging than Pioneer Brand 3165, the most lodging-susceptible commercial check. Six of the seven testcrosses had significantly lower ear height than DeKalb Hybrid 689 and Pioneer Brand 3165. No testcross had a plant height that was significantly greater than that for LH132 × LH51, the shortest commercial check. Testcross entry 1184-1 × FR992/FR1064 yielded 0.13 t ha^{-1} more than the highest yielding commercial hybrid combined with grain moisture and percent erect plants that were within the ranges for the commercial hybrids. This entry also reached midsilk 0.4 days before LH132 × LH51, the earliest-flowering commercial hybrid. Testcross entry 1191-3 × FR992/FR1064 had the highest yield in the experiment in addition to a grain moisture content that was equal to that of DeKalb Hybrid 689. This entry reached midsilk 0.1 days before LH132 × LH51.

For comparison, the three-way cross NC262A × FR992/FR1064 was included in the final year of yield

Table 4 Agronomic trait means for selected Experiment #1 testcrosses and commercial hybrids evaluated in nine North Carolina environments

Entry	Pedigree[a]	Yield (t ha^{-1})	Moisture (%)	Lodging (%)	Ear height (cm)	Plant height (cm)	Days to silk[b]
1239-2	NC262A × 7995-1 F_5S_2 × T	6.49	17.4	27.8	91.0	259.9	74.2
1240-3	NC262A × 7995-1 F_5S_2 × T	6.57	17.1	26.1	86.8	253.8	72.5
1251-1	7995-1 × NC262A F_5S_2 × T	6.76	17.2	26.2	93.4	259.9	75.0
1251-2	7995-1 × NC262A F_5S_2 × T	6.74	17.2	26.0	90.4	260.3	74.7
1251-3	7995-1 × NC262A F_5S_2 × T	6.95	17.7	24.3	89.3	258.9	73.9
1253-4	7995-1 × NC262A F_5S_2 × T	6.79	18.3	23.7	89.8	252.4	73.4
1261-2	7995-1 × NC262A F_5S_2 × T	6.96	18.1	28.7	93.3	249.8	73.0
DeKalb Hybrid 689	Commercial hybrid	6.99	18.0	33.9	108.0	262.9	76.6
LH132 × LH51	Commercial hybrid	7.01	16.8	26.5	98.8	263.6	74.9
Pioneer Brand 3165	Commercial hybrid	6.99	20.1	38.5	106.1	274.4	78.9
Experiment mean		6.83	17.8	28.2	94.7	259.6	74.7
Check mean		7.00	18.3	33.0	104.3	267.0	76.8
LSD 0.05		0.43	0.7	6.4	5.1	5.5	0.9
CV %		6.77	4.0	24.4	5.8	2.2	0.3

[a] T = FR992/FR1064
[b] Flowering data were collected at Clayton only

Table 5 Agronomic trait means for selected Experiment #2 testcrosses and commercial hybrids evaluated in nine North Carolina environments

Entry	Pedigree[a]	Yield (t ha^{-1})	Moisture (%)	Lodging (%)	Ear height (cm)	Plant height (cm)	Days to silk[b]
1180-1	NC262A × 7846-1 F_5S_2 × T	6.98	18.0	28.3	90.8	249.5	74.0
1184-1	NC262A × 7846-1 F_5S_2 × T	7.34	18.5	30.3	89.4	244.1	74.0
1187-1	NC262A × 7846-1 F_5S_2 × T	7.12	17.8	30.2	87.6	244.7	73.7
1191-2	NC262A × 7846-1 F_5S_2 × T	7.07	17.5	31.3	93.3	259.4	74.4
1191-3	NC262A × 7846-1 F_5S_2 × T	7.35	17.9	36.6	98.8	263.0	74.3
1195-1	7846-1 × NC262A F_5S_2 × T	7.05	18.4	35.6	92.5	243.9	75.9
1195-2	7846-1 × NC262A F_5S_2 × T	7.05	18.4	30.9	95.0	248.6	76.1
DeKalb Hybrid 689	Commercial hybrid	6.97	17.9	32.3	103.6	261.8	77.6
LH132 × LH51	Commercial hybrid	7.20	16.7	25.0	97.7	258.8	74.4
Pioneer Brand 3165	Commercial hybrid	7.21	20.3	34.7	103.9	265.7	78.7
Experiment mean		7.13	18.1	31.5	95.3	254.0	75.3
Check mean		7.13	18.3	30.7	101.7	262.1	76.9
LSD 0.05		0.46	0.6	6.8	5.4	5.9	0.8
CV %		6.83	3.7	22.9	6.0	2.5	1.1

[a] T = FR992/FR1064
[b] Flowering data were collected at Clayton only

trials. Ten of the 14 experimental entries out-yielded this check, although not significantly at the $P = 0.05$ level (Table 6). Testcrosses involving lines 1187-1 and 1184-1 had grain yields that exceeded the yield for NC262A × FR992/FR1064 by approximately 0.55 to 0.63 t ha^{-1} (9.5 to 10.9%). The agronomic data, by itself, suggested that some tropical germplasm was probably retained in these lines and that this germplasm combined well with the Stiff Stalk tester. The mean grain-moisture content for the experimental testcrosses was 19.4%, while that for the NC262A × FR992/FR1064 cross was 19.3%. No testcross had a grain moisture that was significantly different from that for DeKalb Hybrid 714. Four experimental entries flowered significantly later than the cross NC262A × FR992/FR1064. None flowered significantly earlier.

SSR experiment

From a set of 159 SSR primer pairs, 62 were selected that produced clear and readily interpretable polymorphisms between NC262A and the all-tropical line 7846-1. Sixty nine primer pairs were selected that revealed polymorphic bands between NC262A and 7995-1. Statistical tests (Bernardo et al. 1997) were applied to subsets of complete SSR data sets. To reduce the potential bias due to linkage relationships and co-inheritance of closely linked markers, only one marker from each chromosome bin position (approximately 20-cM region) that was represented was considered. Marker map locations were obtained from the website of the United States Department of Agriculture/Agricultural Research Service maize genetics database (http://www.agron.missouri.edu/ssr.html). The reduced data set for lines from the NC262A × 7846-1 pedigree had 47 SSR loci, while that for lines from the NC262A × 7995-1 pedigree had 49 SSR loci. The number of SSR

Table 6 1999 agronomic trait means for selected Experiment #1 and #2 testcrosses, and commercial hybrids evaluated in three North Carolina environments

Entry	Pedigree[a]	Yield (t ha^{-1})	Moisture (%)	Lodging (%)	Ear height (cm)	Plant height (cm)	Days to silk[b]
1180-1/94	NC262A × 7846-1 F_5S_2 × T	6.07	19.6	60.7	98.9	257.2	75.0
1184-1/94	NC262A × 7846-1 F_5S_2 × T	6.42	19.3	62.2	98.9	250.0	75.3
1187-1/94	NC262A × 7846-1 F_5S_2 × T	6.34	19.7	57.1	94.4	251.7	75.3
1191-2/94	NC262A × 7846-1 F_5S_2 × T	5.67	19.3	65.6	100.6	263.3	75.3
1191-3/94	NC262A × 7846-1 F_5S_2 × T	6.11	19.3	65.6	105.0	267.8	75.0
1195-1/94	7846-1 × NC262A F_5S_2 × T	6.08	19.5	75.6	96.1	238.9	77.0
1195-2/94	7846-1 × NC262A F_5S_2 × T	5.96	20.0	67.3	101.1	249.4	77.0
1239-2/94	NC262A × 7995-1 F_5S_2 × T	5.59	19.0	58.0	94.4	259.4	75.3
1240-3/94	NC262A × 7995-1 F_5S_2 × T	5.59	18.8	59.9	88.9	253.9	75.0
1251-1/94	7995-1 × NC262A F_5S_2 × T	5.67	19.4	61.0	94.4	256.1	76.3
1251-2/94	7995-1 × NC262A F_5S_2 × T	5.85	18.7	54.4	94.4	260.0	76.3
1251-3/94	7995-1 × NC262A F_5S_2 × T	6.10	19.1	49.2	96.7	260.6	75.7
1253-4/94	7995-1 × NC262A F_5S_2 × T	5.90	19.5	49.2	91.7	255.0	74.7
1261-2/94	7995-1 × NC262A F_5S_2 × T	5.99	20.3	52.9	92.8	250.6	75.0
DeKalb Hybrid 689	Commercial hybrid	5.87	18.8	67.0	111.7	263.3	78.7
DeKalb Hybrid 714	Commercial hybrid	6.65	19.6	47.3	107.2	268.9	76.7
LH132 × LH51	Commercial hybrid	6.20	18.4	55.1	102.8	260.6	76.3
NC262A × FR992/FR1064	Check	5.79	19.3	58.4	92.8	253.9	74.3
Pioneer Brand 3165	Commercial hybrid	5.96	21.3	66.9	106.7	268.3	81.7
Pioneer Brand 32K61	Commercial hybrid	6.63	19.1	38.9	98.9	275.0	77.7
Expt. mean		6.02	19.4	58.6	98.4	258.2	76.2
LSD 0.05		0.96	1.1	16.0	8.5	9.6	1.5
CV %		11.61	3.8	21.1	8.3	3.6	1.2

[a] T = FR992/FR1064
[b] Flowering data were collected at Clayton only

Table 7 Parental contribution estimates for selected F_5S_5 lines

Line	Pedigree	Total # SSR loci[a]	Ratio[b]	p[c]	p 95% CI[d]
1180-1	NC262A × 7846-1 F_5S_5	47	31:16:0	0.68	(0.55, 0.83)*
1184-1	NC262A × 7846-1 F_5S_5	47	15:32:0	0.30	(0.15, 0.46)*
1187-1	NC262A × 7846-1 F_5S_5	47	23:23:1	0.49	(0.30, 0.67)
1191-2	NC262A × 7846-1 F_5S_5	47	24:22:1	0.48	(0.32, 0.66)
1191-3	NC262A × 7846-1 F_5S_5	47	24:22:1	0.53	(0.33, 0.71)
1195-1	7846-1 × NC262A F_5S_5	47	24:21:2	0.54	(0.36, 0.71)
1195-2	7846-1 × NC262A F_5S_5	47	26:21:0	0.55	(0.42, 0.69)
1239-2	NC262A × 7995-1 F_5S_5	49	23:25:1	0.51	(0.30, 0.70)
1240-3	NC262A × 7995-1 F_5S_5	49	22:26:1	0.51	(0.31, 0.70)
1251-1	7995-1 × NC262A F_5S_5	49	26:22:1	0.61	(0.41, 0.81)
1251-2	7995-1 × NC262A F_5S_5	49	26:22:1	0.57	(0.41, 0.73)
1251-3	7995-1 × NC262A F_5S_5	49	20:26:2	0.49	(0.34, 0.65)
1253-4	7995-1 × NC262A F_5S_5	49	23:26:0	0.44	(0.32, 0.56)
1261-2	7995-1 × NC262A F_5S_5	49	23:26:0	0.50	(0.32, 0.70)

*Significantly different from expected based on pedigree at the 0.05 probability level
[a] The total # indicated in the Table is a subset of the complete SSR data set. Statistical tests were applied to subsets in order to reduce potential bias due to SSR linkage relationships
[b] Ratio = (# loci homozygous temperate : # loci homozygous tropical : # heterozygous loci)
[c] p = estimated genetic contribution from temperate parent
[d] p 95% CI = 95% confidence interval for P (Bernardo et al. 1997)

markers included per chromosome in each subset ranged from three to seven.

Each line had a unique SSR genotype. The expected frequency of heterozygous marker loci within each F_5S_5 line was calculated to be 0.0078 (Hallauer and Miranda 1988). The observed frequency of heterozygous loci in the reduced data sets was 0.0179. Values of p (estimated genetic contribution from the temperate parent) ranged from 0.30 to 0.68 with an average value of 0.51 (Table 7). Only two of the 14 lines had genotypes that deviated significantly from that which was expected based on pedigree. Line 1184-1 had a significantly higher proportion of tropical SSR loci (0.70). This is noteworthy since this line was the second highest yielding in testcrosses and out-yielded (although not significantly) three commercial checks after 3 years of testing.

The results indicated that there was very limited overall selection against germplasm from the tropical parents. These marker-based parental contribution estimates are similar to those obtained by other researchers

studying lines derived from bi-parental crosses between U.S. materials (Lorenzen et al. 1995; Bernardo et al. 1997; Bernardo and Kahler 2001). Incorporation was probably aided by the fact that second-cycle all-tropical lines were used as parents in this study. These lines were the result of approximately 13 years of selection to adapt germplasm derived from tropical hybrids to the southeastern United States. Estimates of exotic parental contributions may be less if exotic parents, such as relatively unimproved Latin American landraces, are used as in the GEM Project (Salhuana et al. 1994) or in studies such as that of Holland and Goodman (1995). Five generations of sib-mating were also completed prior to selfing in our study. Results may have been different if selfing was initiated directly out of F_2 populations. Brown (1982) observed that lines extracted from exotic × adapted F_2 populations frequently largely resembled one of the parental strains in appearance. Several workers have emphasized the importance of sib-mating prior to selfing in such populations to reduce the size of unfavorable linkage blocks and increase the formation of desirable recombinants (Nelson 1972; Lonnquist 1974; Geadelmann 1984). On the other hand, sib mating provides for an increased number of generations during which selection against exotic chromosome segments can occur. More comprehensive experiments would be required to address the possible impact of these factors.

Discussion

This experiment resulted in the identification of a small set of lines whose testcrosses compared favorably with several commercial check hybrids for yielding ability, grain moisture content and lodging resistance. In conjunction with molecular-marker data, these results demonstrated that tropical maize germplasm can be incorporated at high rates into a temperate line via pedigree methods in order to derive new inbred lines with acceptable agronomic performance. The distribution of the estimated parental genetic contributions for these lines was similar to those observed for lines derived from temperate × temperate crosses (Bernardo et al. 1997; Bernardo and Kahler 2001).

This project is part of a greater effort to develop inbred lines possessing high amounts of exotic germplasm (Goodman 1985, 1992; Goodman et al. 2000). Methods to make use of exotic germplasm in this program differ from those proposed by other researchers. When an elite parent has more loci containing favorable alleles than an exotic parent, Dudley (1982) stressed the importance of one or more generations of backcrossing to the elite parent in order to have a reasonable probability of extracting improved lines. Selig et al. (1999) suggested that the amount of exotic germplasm incorporated into elite maize inbreds should be relatively small if useful improvements are desired, because "introduction of too much DNA from exotic germplasm would probably adversely alter elite hybrid maize genomes." The 'advanced QTL-backcross' method is a molecular-marker-based approach for simultaneous identification of favorable exotic quantitative trait loci (QTLs) and their transfer to elite material (Tanksley and Nelson 1996; Bernacchi et al. 1998). Stuber et al. (1999) proposed a near-isogenic line-approach for transferring chromosome segments from exotic sources. In this method, small segments from an exotic donor source are backcrossed into elite lines of maize without prior identification of QTLs. A collection of lines is generated, each line possessing a different chromosome segment from the exotic parent. Near-isogenic lines are then evaluated in combination with an appropriate tester for quantitative traits such as yield. Superior lines are presumed to have received a favorable QTL from the exotic source.

These methods would be expected to introduce small amounts of exotic germplasm, but do little to broaden the germplasm base. It is possible that they would result in improved inbred lines, but they fail to fully exploit exotic source material. Negative aspects of QTL-type approaches include the extensive phenotypic evaluation that is required to obtain useful data and the low likelihood of detecting chromosomal regions with small effects (Beavis 1998). Any procedures that involve backcrossing are likely to result in random loss of favorable exotic alleles during the process (Crossa 1989). Favorable epistatic interactions from the exotic parent are also unlikely to be transferred by using these methods.

Approaches that more adequately satisfy long-term goals are those that boost genetic variability, extend selection limits beyond current levels and increase flexibility in breeding for a range of desired outcomes. The work of Goodman (1985, 1992) and Goodman et al. (2000) places increased priority on meeting these objectives and has adopted the approach of initially adapting 100%-tropical populations to the Southeastern United States. This germplasm is not mixed with U.S. materials until it begins to provide a flow of adapted lines with increased agronomic performance (Holley and Goodman 1988b; Moreno-Mendoza 1989; Uhr and Goodman 1995). In contrast to methods that involve backcrossing, this approach permits incorporation of large amounts of exotic germplasm into elite U.S. lines. This approach may require greater patience but, with prudent choice of exotic source material, it should result in a stream of lines possessing high amounts of divergent genes for yield and disease resistance.

Acknowledgements This work was supported in part by Pioneer Hi-Bred International, Inc., through a graduate fellowship provided to the senior author.

References

Bailey TB Jr (1977) Selection limits in self-fertilizing populations following the cross of homozygous lines. In: Pollak E, Kempthorne O, Bailey TB Jr (eds) Proc Int Conf on Quantitative Genetics, Iowa State University Press, Ames, Iowa, pp 399–412

Bailey TB Jr, Comstock RE (1976) Linkage and the synthesis of better genotypes in self-fertilizing species. Crop Sci 16:363–370

Beavis WD (1998) QTL analysis: power, precision, and accuracy. In: Paterson AH (ed) Molecular dissection of complex traits. CRC Press, Boca Raton, Florida, pp 145–162

Bernacchi D, Beck-Bunn T, Emmatty D, Eshed Y, Inai S, Lopez J, Petiard V, Sayama H, Uhlig J, Zamir D, Tanksley S (1998) Advanced backcross QTL analysis of tomato. II. Evaluation of near-isogenic lines carrying single-donor introgressions for desirable wild QTL-alleles derived from *Lycopersicon hirsutum* and *L. pimpinellifolium*. Theor Appl Genet 97:170–180

Bernardo R, Kahler AL (2001) North American study on essential derivation in maize: inbreds developed without and with selection from F_2 populations. Theor Appl Genet 102:986–992

Bernardo R, Murigneux A, Maisonneuve JP, Johnsson C, Karaman Z (1997) RFLP-based estimates of parental contribution to F_2- and BC_1-derived maize inbreds. Theor Appl Genet 94:652–656

Brown WL (1982) Exotic germplasm in cereal crop improvement. In: Vasil IK, Snowcroft WR, Frey KJ (eds) Plant improvement and somatic cell genetics. Academic Press, Incorporated, New York, pp 29–42

Cochran WG, Cox GM (1957) Experimental designs. John Wiley and Sons, Incorporated, New York

Crossa J (1989) Theoretical considerations for the introgression of exotic germplasm into adapted maize populations. Maydica 34:53–62

Dudley JW (1982) Theory for transfer of alleles. Crop Sci 22:631–637

Dudley JW (1984) A method for identifying lines for use in improving parents of a single cross. Crop Sci 24:355–357

Duvick DN (1992) Genetic contributions to advances in yield of U.S. maize. Maydica 37:69–79

Geadelmann JL (1984) Using exotic germplasm to improve northern corn. Proc Annu Corn Sorghum Res Conf 39:98–110

Godshalk EB, Kauffmann KD (1995) Performance of exotic × temperate single-cross maize hybrids. Crop Sci 35:1042–1045

Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research, 2nd edn. John Wiley and Sons, Inc, New York

Goodman MM (1985) Exotic maize germplasm: status, prospects, and remedies. Iowa State J Res 59:497–527

Goodman MM (1992) Choosing and using tropical corn germplasm. Proc Annu Corn Sorghum Res Conf 47:47–64

Goodman MM, Brown WL (1988) Races of corn. In: Sprague GF, Dudley JW (eds) Corn and corn improvement. American Society of Agronomy, Madison, Wisconsin, pp 33–79

Goodman MM, Carson ML (1999) Developing temperate inbreds from tropical germplasm: rationale, results, and conclusions. Proc Annu Illinois Corn Breeders School 35:1–19

Goodman MM, Castillo F, Moreno J (1990) Choosing and using exotic maize germplasm. Proc Annu Illinois Corn Breeders School 26:148–171

Goodman MM, Moreno J, Castillo F, Holley RN, Carson ML (2000) Using tropical maize germplasm for temperate breeding. Maydica 45:221–234

Hallauer AR (1978) Potential of exotic germplasm for maize improvement. In: Walden DB (ed) Maize breeding and genetics. John Wiley and Sons, New York, pp 229–247

Hallauer AR, Miranda Fo JB (1988) Quantitative genetics in maize breeding. Iowa State University Press, Ames, Iowa

Holland JB, Goodman MM (1995) Combining ability of tropical maize accessions with U.S. germplasm. Crop Sci 35:767–773

Holland JB, Uhr DV, Jeffers D, Goodman MM (1998) Inheritance of resistance to southern corn rust in tropical by corn-belt maize populations. Theor Appl Genet 96:232–241

Holley RN, Goodman MM (1988a) Stalk quality and stalk rot resistance of tropical hybrid maize derivatives. Plant Dis 72:321–324

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

Holley RN, Goodman MM (1989) New sources of resistance to southern corn leaf blight from tropical hybrid maize derivatives. Plant Dis 73:562–564

Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) SAS system for mixed models. SAS Institute Inc, Cary, North Carolina, USA

Lonnquist JH (1974) Considerations and experiences with recombinations of exotic and corn belt maize germplasms. Proc Annu Corn Sorghum Res Conf 29:102–117

Lorenzen LL, Boutin S, Young N, Specht JE, Shoemaker RC (1995) Soybean pedigree analysis using map-based molecular markers. I. Tracking RFLP markers in cultivars. Crop Sci 35:1326–1336

Lu H, Bernardo R (2001) Molecular marker diversity among current and historical maize inbreds. Theor Appl Genet 103:613–617

McIntosh MS (1983) Analysis of combined experiments. Agron J 75:153–155

Moreno-Mendoza JD (1989) Temperate and tropical evaluation of temperate maize lines derived from totally photosensitive materials. PhD dissertation, North Carolina State University, Raleigh, North Carolina. (Dissertation Abstract 90-06638)

Nelson HG (1972) The use of exotic germplasm in practical corn breeding programs. Proc Annu Corn Sorghum Res Conf 27:115–118

Riede CR, Anderson JA (1996) Linkage of RFLP markers to an aluminum tolerance gene in wheat. Crop Sci 36:905–909

Salhuana W, Pollak L, Tiffany D (1994) Public/private collaboration proposed to strengthen quality and production of USA corn through germplasm enhancement. Diversity 10:77–78

Selig L, Lambert RJ, Rocheford TR, da Silva WJ (1999) RFLP and cluster analysis of introgression of exotic germplasm into U.S. maize inbreds. Maydica 44:85–92

Senior ML, Chin ECL, Lee M, Smith JSC, Stuber CW (1996) Simple sequence repeat markers developed from maize sequences found in the GENBANK database: map construction. Crop Sci 36:1676–1683

Simmonds NW (1993) Introgression and incorporation. Strategies for the use of crop genetic resources. Biol Rev 68:539–562

St Martin SK (1982) Effective population size for the soybean improvement program in maturity groups 00 to IV. Crop Sci 22:151–152

Stuber CW (1978) Exotic sources for broadening genetic diversity in corn breeding programs. Proc Annu Corn Sorghum Res Conf 33:34–47

Stuber CW, Polacco M, Senior ML (1999) Synergy of empirical breeding, marker-assisted selection, and genomics to increase crop yield potential. Crop Sci 39:1571–1583

Tallury SP, Goodman MM (1999) Experimental evaluation of the potential of tropical germplasm for temperate maize improvement. Theor Appl Genet 98:54–61

Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92:191–203

Troyer AF (1999) Background of U.S. hybrid corn. Crop Sci 39:601–626

Troyer AF (2001) Temperate corn – background, behavior, and breeding. In: Hallauer A (ed) Specialty corns, 2nd edn. CRC Press, Boca Raton, Florida, pp 393–466

Uhr DV (1991) Adaptation of tropical maize to temperate zones. PhD dissertation, North Carolina State University Raleigh, North Carolina. (Dissertation Abstract 92-03177)

Uhr DV, Goodman MM (1995) Temperate maize inbreds from tropical germplasm. I. Testcross yield trials. Crop Sci 35:779–784