COMBINING ABILITY OF A TROPICAL-DERIVED MAIZE POPULATION WITH ISOCENIC BT AND CONVENTIONAL TESTERS

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ABSTRACT • Bt transgenes, which confer protection against lepidopteran pests, have been introduced widely into commercial breeding populations. If inbred lines exhibit specific combining ability interactions between conventional and Bt testers, separate breeding programs for inbred lines to be used in conventional and transgenic hybrids may be warranted, complicating current hybrid breeding procedures. This possibility was investigated by testing crosses of 97 F2 plants from the cross of two tropical-derived inbred lines, NC296 and NC296, to both isogenic Bt and conventional (non-Bt) hybrids. The tester hybrids were either identical to or closely related to hybrids commercialized in the U.S.A. The 97 Bt testcrosses and 97 non-Bt testcrosses were evaluated in replicated yield trials in seven North Carolina environments along with four commercial checks and the two hybrids per se. The Bt testcrosses had slightly lower mean grain moisture (0.2%) and plant height (0.05 m) than the non-Bt testcrosses, but otherwise did not differ from them. No F1 by tester interactions were observed for any trait, and genetic correlations between the same trait measured on different testers were high (r2 = 0.85 for grain yield measured on Bt and non-Bt testers). These results suggest that use of Bt and conventional testers will give similar results, simplifying the use of Bt transgenics in maize breeding programs. Whereas Bt transgenics did not improve yield, tropical F2 testcrosses had greater grain yields than commercial hybrids, suggesting that greater increases in maize yield potential are likely to result from exploitation of exotic maize germplasm than from single gene transformations.

KEY WORDS: Transgenic; Maize breeding; Tropical germplasm.

INTRODUCTION

Transfer of transgenes coding for insecticidal proteins originally obtained from the bacterium Bacillus thuringiensis (Bt genes) into commercial maize hybrids has been a high priority for seed companies. Some Bt genes are highly effective at protecting the plant from damage by European corn borer (Ostrinia nubilalis) (Hubner) larvae (Kozel et al., 1993). European corn borer causes yield losses estimated to exceed $1000 million per year (Mason et al., 1996). European corn borer frequently attacks maize grown in the coastal plain region of North Carolina, and the second generation larvae begin feeding around the onset of flowering of most maize hybrids in this region (Sorensen and Bazer, 1994). Maize hybrids containing Bt genes have been rapidly adopted in the United States, accounting for 20% of the crop area in 1999, although this proportion decreased to 19% in 2000 (Carpenter and Given, 2001). Introduction of transgenes into crops causes genomic changes beyond the addition of a new gene to a chromosome. The transgene can integrate into a functional gene or its regulatory sequence, disrupting its function or expression. In addition, heritable genomic changes associated with somaclonal variation also accompany maintenance of plants as tissue cultures during the transformation process. Somaclonal variations may include point mutations, chromosomal structural changes, somatic crossovers, DNA amplification and deletions, and activation of transposable elements (Larkin and Scowcroft, 1981). Somaclonal variation in quantitatively inherited agronomic traits of maize, including grain yield and grain moisture, was reported by Lee et al. (1988).

Genetic differences caused by a transgene and its insertion into the genome may affect the breeding value of a genotype even for traits not directly related to the transgene’s primary product. In the case of Bt maize lines, their breeding value for important agronomic traits, such as grain yield, flowering time, height, and lodging resistance may be af-
ected in uncertain ways. Similarly, pleiotropic effects of transgenes can affect the performance of transgenic lines or hybrids as testers for evaluating the combining ability of lines developed from conventional breeding programs.

A long-term objective of the maize breeding program at North Carolina State University is to develop tropical maize germplasm adapted to temperate corn production environments as an alternative heterotic group for breeding in the United States. The breeding value of tropical breeding lines for use in the temperate USA is assessed by their combining ability with testers representing important Corn Belt Dent germplasm. Previous research indicates that most tropical germplasm combines equally well with Reid and Lancaster testers, which represent the dominant heterotic groups of the United States maize crop (Holland and Goodman, 1995). This simplifies breeding tropical maize germplasm for use in hybrids with Corn Belt Dent germplasm, because tropical breeding lines can be satisfactorily evaluated for general combining ability with one hybrid tester, at least during preliminary screening stages.

Does the introduction of a Bt gene into a hybrid alter its ability as a tester to discriminate among tropical germplasm lines? The Bt gene itself could affect evaluation of tropical lines, because the natural variation for resistance to European corn borer feeding that may exist among breeding lines would be masked. As this resistance can indirectly affect grain yield, then breeding values for grain yield may be affected. Other genomic changes that accompany the transformation process could also affect a hybrid’s function as a tester in ways that cannot be easily predicted. The objective of this experiment was to compare isogenic conventional and Bt hybrids as testers for evaluating early generation families of a cross between two temperate-adapted inbred lines of tropical origin.

MATERIALS AND METHODS

Experimental Population

An F1 population was created from the cross of inbred maize lines NC296 and NC298. NC296 and NC298 are both 100% tropical germplasm lines that are adapted to temperate environments. The lines were developed at North Carolina State University from crosses among tropical double-cross hybrids (Teague and Goodman, 1999). Ninety-seven F2 plants were tested for isogenic Syngenta hybrids that presumably differed only by the presence or absence of a Bt gene. The Bt tester hybrid carried two alleles of the Bt construct; therefore, all testers progeny made with the Bt tester were heterozygous for the Bt transgene. Room et al. (1993) described the development of the original transgenic line from which the Bt hybrid was developed. The Bt transgene is a synthetic CryIA(b) construct and the Bt hybrid was developed from transformation event 176. The Bt tester used in this experiment was not commercially marketed, but a closely related hybrid homozygous for the transgene was marketed as Gua brand hybrid "MAX XV". The conventional isogenic tester was marketed as Gua brand hybrid "4404". The genetic background of these isogenic hybrids represents an improved 375 x non-traditional-stalk type (C. Foils, Syngenta, pers. comm.).

Experimental Design

Plants of F2 testcross families were assigned at random to one of four experimental sets. Each set contained 24 pairs of F2 testcross families, except set 4, which had 25 pairs. Sets 1, 2, and 3 also contained the following check entries: the Bt hybrid, the isogenic non-Bt hybrid, the F1 generation of NC296 x NC298 x Bt hybrid, the F1 generation of NC296 x NC298 x non-Bt hybrid, Pioneer brand hybrids 3516S and 356321, and Dekalb brand hybrids 689 and 711. Set 1 contained the same checks except Pioneer brand hybrid 356321 and Dekalb brand hybrid 689. Thus, a total of 56 entries was included in each set.

The experimental design was a replications within sets design, with each set arranged as a 7 x 4 lattice design replicated two or three times at each of seven environments. Testing environments were the Central Crops Research Station in Clayton, the Peanut Belt Research Station in Lewiston, and the Tidewater Research Station in Plymouth in 1993; and the same three sites plus the Sand Hill Research Station in Jackson Springs in 2000. Experiments were replicated three times at each location in 1999 and twice at each location in 2000.

At all locations, plots consisted of two 1346 m rows down with 44 seeds, including a 0.240 meter length alley at the end of each plot. In Plymouth and Clayton, rows were spaced 0.97 m apart, resulting in a target population density within the planted area of the plots of 56,680 plants ha-1. In Lewiston and Jackson Springs, inter-row spacing was 0.91 m, and target population density was 59,400 plants ha-1. Recommended planting densities for average soil types in North Carolina range from 54,300 to 59,500 plants ha-1 (Hinson and Brown, 2000).

During the growing season, the number of plants, mean plant height, and mean height to node of topmost ear (ear height) were recorded for each plot at every location. Days to anthesis and silking were recorded only at Clayton in both years. Anthesis date was the first date on which at least 50% of the plants in the plot were shedding at least 50% of the available pollen. Silk emergence first date was the date on which 90% of the plants in the plot were displaying visible silks. Numbers of root-lodged plants and stalk-lodged plants were counted at every location except Lewiston and Plymouth in 1999. Plants lodging at an angle of 90° or more were counted as root lodged if the stalk was not broken. Plants with stalks broken below the ear or with dropped ears were counted as stalk lodged. In 1999 hurricanes Dennis and Floyd made landfall on the coast of North Carolina before harvest and resulted in severe lodging. Therefore a visual estimate of percent erect plants was performed at the coastal plains locations (Plymouth and Lewiston) in 1999. At maturity, 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

Percent stand was estimated from each plot as the number of plants per plot divided by 10, the desired number of plants per plot. For environments in which the number of root and stalk lodged plants was recorded, the percentage of erect plants for each plot was the proportion of plants neither root nor stalk lodged.

Within each combination of set and environment, data was analyzed using Proc MIXED in SAS, version 8.0 (SAS Institute Inc., 1999). Each experimental or cross hybrid was considered a fixed factor, whereas replication and incomplete lattice block within replication were treated as random factors. Entry was considered a fixed factor because, although the F_{1} testcross families were a random sample from the NC296 x NC298 population, the check hybrids were chosen to represent the best available cultivars and the test hybrid were not representative of any definable reference population. Percent stand was used as a covariate for grain yield only. Entry least square means adjusted for incomplete block effects were estimated from each set and environment combination.

To compare the distributions of check entries and family testcross means on different testers, entry means from each set and environment were analyzed with Proc MIXED in SAS, considering entry to be a fixed effect and environment, set, environment by set interaction, and entry by set interaction to be random factors. Only the check entries that were included in three of four sets contributed to the entry by set interaction. Entry by environment and entry by environment by set interactions contributed to the residual variance of this model. Least square means of entries were computed from this model, which resulted in their being adjusted for set effects.

To test for family by tester interactions, a subset of the data was used, which included NC296 x NC298 testcross family means from each set and environment, but lacked all check entries. These data were analyzed with Proc MIXED in SAS, considering tester to be a fixed effect and environment, set, environment by set interaction, tester by set interaction, tester by set by environment, family within set, environment by family within set, and tester by family within set to be random factors. Proc MIXED estimates variance components of random factors, which cannot be tested with F-testistics, as is done with traditional analysis of variance. Appropriate significance tests of random factors in Proc MIXED are achieved by chi-square tests of the difference between the -2 restricted maximum likelihood (REML) log-likelihoods of the complete model and a reduced model lacking the random factor in question (Littell et al., 1996). The approximate p-value of the test can be obtained by dividing the p-value of the one degree of freedom chi-square statistic by two (Littell et al., 1996, SED and Liang, 1987). By deleting the tester by family within set factor from the model, reanalyzing the data, and computing the appropriate chi-square value, we tested the significance of the tester by family within set interaction for each variable.

The same subset of data lacking check entries was used to estimate heritabilities and genotypic and phenotypic correlations between the same trait measured on different testers. Multivariate analysis of variance (MANOVA) was performed for each agronomic trait measured on the two testers, considering them to be different variables. For example, grain yield measured on testers to the F_{1} tester and on testcrosses to the non-F_{1} tester were considered different variables, and their genotypic and phenotypic correlations were estimated. Genotypic and phenotypic by environmental covance components between each trait measured on different testers were estimated using restricted maximum likelihood (REML) in SAS Proc MIXED. Multivariate REML estimates were obtained with Proc MIXED by treating each pair of variables as repeated measurements of a single variable at each location (Winston, 1998). MANOVAS were performed with SAS Proc MIXED, including environment, set, environment by set interaction, and family within set as random factors in the model. Environment by family within set interaction contributed to the residual variance; in this analysis, because it was based on entry means at each environment. The genetic correlation was estimated as:

\[ r_{g} = \frac{\hat{\sigma}_{G12}}{\sigma_{G1} \sigma_{G2}} \]

where \( \hat{\sigma}_{G12} \) is the estimated genotypic covariance between traits 1 and 2 and \( \sigma_{G1} \) is the estimated genotypic standard deviation for trait 1 (i = 1 or 2). The phenotypic correlation on a family mean basis was estimated as:

\[ r_{p} = \frac{\hat{\sigma}_{P12}}{\sigma_{P1} \sigma_{P2}} = \frac{\sigma_{G12} + \sigma_{GEM2}}{\sqrt{\sigma_{G1}^2 + \sigma_{GEM1}^2}} \cdot \frac{\sqrt{\sigma_{G2}^2 + \sigma_{GEM2}^2}}{e} \]

where \( \sigma_{P12} \) and \( \sigma_{GEM1,2} \) are the phenotypic covariance and genotype by environment (confounded with residual) covariance estimates, respectively, between traits 1 and 2, \( \sigma_{P1} \) and \( \sigma_{P2} \) are the phenotypic and genotype by environment (confounded with residual) standard deviation estimates for trait 1, and \( e \) is the number of environments from which the family means were computed (e = 2 for anthesis and silk dates, e = 7 for other traits). Approximate sampling variances for the heritability, genotypic correlation, and phenotypic correlation estimates were obtained using the delta method (Halvorson et al., 1993; Linan and Winston, 1998). Matrix computations necessary to obtain the standard errors were performed using SAS Proc IML (Holand et al., 2005; SAS Institute Inc., 1999).

RESULTS AND DISCUSSION

Final stands averaged 92% of target population densities, resulting in mean actual densities of 52,321 to 55,230 plants ha\(^{-1}\). Mean stands varied among entries from 73% to 100% of target population densities, therefore, grain yields were adjusted to mean actual population density by using stand as a covariate. Significant (P < 0.05) variation was observed among entries for all traits. The mean grain yield, grain moisture, days to anthesis and silk, and ear and plant heights of NC296 x NC298 F_{2} testcrosses to either tester were significantly (P < 0.05) greater than both hybrid testers (Table 1). The mean grain yield and percentage erect plants of the NC296 x NC298 F_{2} testcrosses to either tester were significantly (P < 0.05) greater than the mean of
four commercial hybrids (Table 1). As expected, the NC296 x NC298 F₁ and F₂ generations had essentially the same agronomic performance when crossed to the same tester (Table 1).

The main effect of tester was significant only for grain moisture and plant height (Table 1). The NC296 x NC298 F₂ testcrosses to the Bt tester had lower grain moisture and greater plant height than the testcrosses to the non-Bt tester, but the mean differences were small (0.3% difference in grain moisture and 0.5 m difference in height, Table 1). The same differences were observed in the F₁ testcrosses, but these were not significant because of the limited power to detect differences between individual entries compared to means of 97 entries. The Bt tester per se also had significantly lower grain moisture and lower rank for plant height than the non-Bt tester, consistent with the responses observed in the testcrosses (Table 1). This is in contrast to other researchers' previous experience with Bt hybrids (including event 176), in which the Bt hybrids tended to be later flowering and have greater grain moisture than isogenic conventional hybrids (T. Miller, Cargill Hybrid Seeds, pers. comm.).

Family-by-tester interaction was not significant for any of the variables measured. Furthermore, genetic correlation estimates between a trait measured on Bt testcrosses and the same trait measured on non-Bt testcrosses were high (Table 2). The estimate of the genetic correlation between grain yield measured on the Bt tester and on the non-Bt tester was $r = 0.85$, and other genetic correlation estimates were even higher (Table 2). The phenotypic correlation estimates between pairs of traits measured on
FIGURE 1 - Histogram of 97 NC290 x NC294 F₂ maize testcross family means for grain yield on a Bt tester and non-Bt tester.

FIGURE 2 - Histogram of 97 NC290 x NC298 F₂ maize testcross family means for grain moisture on a Bt tester and non-Bt tester.
### TABLE 3 - Agronomic performance of ten best NC296 x NC298 F2 maize testcross families and individual check hybrids evaluated in seven North Carolina environments.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Grain yield</th>
<th>Grain moisture</th>
<th>Erect plants</th>
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<tr>
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<td>Averaged across testers1</td>
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<td>Non-Bt tester</td>
</tr>
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<td>NC296 x NC298 family 3157</td>
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<td>8.6</td>
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<td>8.2</td>
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<td>8.2</td>
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</tr>
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<td>Dekalb brand hybrid 714</td>
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<td></td>
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<tr>
<td>Pioneer brand hybrid 33X61</td>
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<tr>
<td>Pioneer brand hybrid 3165</td>
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<tr>
<td>Dekalb brand hybrid 689</td>
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<td></td>
<td></td>
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<tr>
<td>Bt tester</td>
<td>6.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Bt tester</td>
<td>6.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>0.5</td>
<td>0.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

1 Means of F2 testcrosses averaged across testers or of commercial hybrids per site.
2 LSD (0.05) to compare an experimental testcross to a check hybrid.

Different testers also tended to be high, but lower than the genetic correlation estimates, because the phenotypic covariances were similar to the genotypic covariances, whereas the phenotypic variances were greater than the genotypic variances due to non-genetic effects (Table 2).

Heritabilities of testcross family means were significant for all traits. Most traits had similar heritabilities when measured on Bt and non-Bt testers (Table 2). However, heritability estimates of grain yield and anthesis date were 20 percentage points smaller on the Bt tester than the non-Bt tester (Table 2). Genetic variance for grain yield was 0.017 ± 0.007 (Mg ha⁻¹)² on the Bt tester and 0.046 ± 0.002 (Mg ha⁻¹)² on the non-Bt tester. Histograms of family means for grain yield on Bt and non-Bt testers illustrate the greater dispersion of the non-Bt testcrosses than the Bt testcrosses (Figure 1). In contrast, the histograms of testcross family means for grain moisture on Bt and non-Bt testers have similar dispersions, but the testcrosses to the non-Bt tester are shifted toward greater mean moistures (Figure 2).

The lack of family by tester interaction, the similarity of family means on isogenic Bt and non-Bt testers, and the high genetic correlations between testcross means on the two testers strongly suggest that testing for specific combining ability interactions with transgenic Bt testers is not necessary to select tropical maize families with superior combining ability with either Bt or non-Bt temperate germplasm. If the top ten Bt testcross families were selected based on grain yield, these would include F2 plants that also made six of the best ten non-Bt testcrosses. Similarly, if the top ten yielding non-Bt testcross families were selected, these would include F2 plants that made four of the best ten Bt testcrosses. Mean grain yields of the superior families tended to be similar, irrespective of the tester used (Table 3).

In summary, few substantial differences were observed between testcrosses with the Bt and non-Bt testers. Testcrosses to the Bt hybrid had lower grain moisture on average (Table 1, Figure 2), but mean grain yields were similar. Genetic variance for grain yield was greater when the non-Bt hybrid was used as a tester, and gain from selection with the non-Bt tester is expected to be greater. Otherwise, however, it seems that the choice of a Bt or non-Bt tester for tropical-derived lines is immaterial. Interferences from our results to all Bt transgenic hybrids may be limited, however, because of variation in hybrid genetic background and effectiveness of Bt transgenes. Van Dyne (2001) observed that the
event 176 transgene used in this experiment exhibited high levels of effectiveness against first generation European corn borer (ECB), moderate levels of effectiveness against second generation ECB, and poor control against third generation ECB in North Carolina. Furthermore, event 176 expresses only very low levels of toxin in ear tissue (Koziri et al., 1993), limiting its effectiveness against second and later generation ECB. Van Duyne (2001) suggested that other Bt transgene constructs and events have greater effectiveness against ECB under North Carolina growing conditions, and our results may have differed if a Bt gene with ubiquitous (rather than tissue-specific) expression were used. In addition, Ritz and Pulizzi (1997) observed that grain yield differences of Bt hybrids compared to their near-isogenic conventional counterparts varied across hybrids and environments (representing different levels of corn borer infestation).

Aside from comparisons of transgenic and conventional testers, our results highlight the potential usefulness of tropical germplasm as a new heterotic group for temperate maize growing regions. The mean of the NC290 x NC298 F2 testcross families was about one Mg ha\(^{-1}\) greater than their temperate hybrid tester. Furthermore, all NC290 x NC298 F2 testcrosses ranked higher for grain yield than both hybrid testers (Figure 1). The Bt hybrid used as a tester in this experiment was not commercialized, but it is closely related to a Bt hybrid that was commercialized (as Ghiu brand “MAX454”). The mean yield of the tropical family testcrosses was also significantly greater than the mean yield of four commercial conventional hybrid checks. The two high-yielding testcross families were significantly superior to the highest-yielding commercial check hybrid (DK714) for grain yield, and also equal or superior in terms of lower grain moisture and greater percentage of erect plants at harvest (Table 3). The mean grain moisture content of the ten highest-yielding tropical testcrosses was 17.7%, which was lower than the highest-yielding hybrid (DK714), but higher by about one percentage point than two other high-yielding commercial hybrids (3ZK61 and 3165). Higher grain moisture at harvest can increase the costs of production if grain must be dried before selling, therefore, selection for reduced grain moisture content in this population is needed. The genetic correlation between grain yield and grain moisture in this population was not significantly different from zero (data not shown), suggesting that selection could effectively lower grain moisture without reducing grain yield, as demonstrated in another tropical maize population by Hawbaker et al. (1997).

The relative impact of the Bt transgene and tropical germplasm can be assessed from this experiment. Introduction of the Bt transgene did not improve grain yield relative to the isogenic non-Bt hybrid. Creating testcross families by crossing the Bt and non-Bt hybrids to random F2s from a cross between two publicly developed tropical inbred lines, however, resulted in about a one Mg ha\(^{-1}\) increase in yield (a 14 percent increase over the non-Bt hybrid mean). Although the Bt gene may improve yield stability by preventing yield losses under corn borer infestations, the Bt gene does not improve yield potential, and did not improve realized yield in this experiment.

Goodman and Carson (2000) suggested that the development of a commercially acceptable maize line carrying a new transgene would cost at least 25 times the development of an agronomically elite exotic maize line representing a new germplasm source. The relative costs of transgenic maize hybrid development to exotic germplasm work seem to be inversely related to their potential to improve productivity of temperate maize.

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REFERENCES


