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Evaluating testcross performance and genetic divergence of lines derived from reciprocal tropical maize composites

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Abstract

The development of hybrids with enhanced expression of heterosis depends on the genetic background of the source population and the effectiveness of a breeding scheme used to identify and use divergent inbred lines with good combining ability. The present study was conducted to examine the potential of improved tropical reciprocal composites as sources of inbred lines for developing productive hybrids. Thirty-six S_4 lines derived from the third RRS cycle of each composite were crossed in pairs to form 36 testcrosses, which were evaluated along with commonly grown commercial hybrids in Nigeria across nine test environments. Results showed consistent ranking of testcrosses for grain yield and other traits across the diverse test environments. The best 22 testcrosses produced 21% to 51% more grain yields than the highest yielding commercial hybrid. Several testcrosses combined high grain yields with other desirable agronomic traits. The SSR and SNP markers used in the present study detected a broad range of genetic diversity among the 72 S_4 lines, which was structured along the two composites. The two markers portrayed similar trends in determining genetic distance estimates and detecting composite-specific alleles in the S_4 lines. These results highlight the potential of improved reciprocal composites as sources of new and divergent parents for developing productive hybrids and as sources of novel alleles for broadening and diversifying the genetic base of adapted germplasm to sustain genetic gain in productivity of hybrids in WCA.

Introduction

Maize has emerged as a dominant staple food crop grown in diverse agro-ecological zones and consumed by millions of people in West and Central Africa (WCA). Both the area planted to maize and grain production have increased significantly in this region as a result of expanded use of the crop for food, animal feed, and industrial products. The introduction of improved maize cultivars adapted to the diverse agro-ecological zones has contributed to the significant increase in maize production in the various countries in WCA (Alene et al, 2009). Most of the area in this region is planted to improved open-pollinated maize varieties (Rusike and Eicher, 1997) mainly because private seed companies are not well developed in many countries. (Auta et al, 2001) suggests that the development and accelerated deployment of maize hybrids can allow greater increases in maize yields in the major maize producing countries in WCA. Studies have demonstrated that hybrids can increase farmers' maize yields by more than 40% in favourable growing environments and by more than 30% even under stressful conditions (Byerlee and Jewell, 1997).

The International Institute of Tropical Agriculture (IITA) started a hybrid breeding program in 1979 to strengthen involvement of the private sector in the production and marketing of hybrid maize in WCA (Kim, 1997). This program focused on generating hybrids with high yield potential and resistance to specific biotic and abiotic stresses for achieving greater and dependable yields in the major production zones in this region (Efron et al, 1989; Kim, 1997). Inbred lines with desirable agronomic and adaptive traits developed from bi-parental crosses, backcrosses, and broad-based populations improved by intra-population selection schemes were used to develop hybrids (IITA, 1992; MIP, 1996). Considering the importance of having hybrid oriented populations and application of selections schemes that maximize expression of heterosis in hybrids, IITA utilized results of diallel studies and promising heterotic patterns of tropical germplasm described by Wellhausen (1978) and Goodman (1985) as the basis to create two late maturing composites known as TZL COMP3 and TZL COMP4 for a long-term reciprocal recurrent selection program following the comprehensive breeding approach proposed by (Eberhart et al, 1967). This approach was adopted because it allowed exploitation of the genetic gains attained using intra-population improvement methods to create the two composites for enhancing efficiency in generating both open-pollinated maize varieties and parental lines of hybrids with better agronomic performance and adaptation to the moist savannas in WCA (IITA 1992; MIP 1996).

The two composites have been subjected to four cycles of reciprocal recurrent selection (RRS) to boost agronomic performance of hybrids formed from in-bred lines derived from advanced selection cycles (Hallauer and Eberhart, 1970; Betran and Hallauer, 1996). As RRS requires three years to complete a cy-cle, assessment of the potential of TZL COMP3 and TZL COMP4 as sources of inbred lines for develop-ing hybrids with superior agronomic performance is important to continue the selection process. Recent studies found reciprocal populations as sources of inbred lines whose single-crosses produced compa-rable or higher grain yields than the best commercial single-cross hybrids (Jenweerawat et al, 2010; Souza Jr et al, 2010). Performance evaluation of specific combinations of lines derived from advanced selec-tion cycles of TZL COMP3 and TZL COMP4 may thus provide an indication on whether the two composites should be retained and improved as sources of new inbred lines with better performance and adaptation to changing climatic conditions (Carena, 2005).

Molecular marker-based diversity assessment of inbred lines derived from advanced selection cycles may also provide an indication about the potential of TZL COMP3 and TZL COMP4 as sources of di-vergent parental lines for crossing to maximize het-erosis in hybrids. Several studies found high levels of genetic divergence between lines derived from ad-vanced selection cycles of reciprocal populations us-ing restricted fragment length polymorphism (RFLP) (Messmer et al, 1991; Labate et al, 1997; Hagdorn et al, 2003; Hinze et al, 2005) and SSR loci (Pinto et al, 2003; Berilli et al, 2011; Romay et al, 2012). Similar results were also reported in reciprocal populations characterized using single nucleotide polymorphism (SNP) markers (Gerke et al, 2013). As the SSR and SNP markers differ in their information content, mu-tational rates, extent of genome coverage, and reli-ability for genotyping (Hamblin et al, 2007; Jones et al, 2007; Ingelandt et al, 2010), combined use of the two classes of markers may provide robust assess-ment of consistency of the genetic diversity of inbred lines derived from advanced selection cycles of TZL COMP3 and TZL COMP4. Several comparative stud-ies in maize found that SSRs did better in separat-ing lines into distinct groups than SNPs although the two classes of markers detected similar genetic structures in diverse inbred lines (Hamblin et al, 2007; Jones et al, 2007; Ingelandt et al, 2010).

The present studies were conducted (i) to com-pare agronomic performance of testcrosses between pairs of S4 lines derived from advanced selection cy-cles of TZL COMP3 and TZL COMP4 with commer-cial hybrids and (ii) to examine the patterns of genetic diversity existing among lines derived from advanced selection cycles of the two composites using SSR and SNP markers.

Materials and Methods

Genetic materials

Diallel crosses of late maturing maize populations were evaluated in a field trial in multiple locations in 1988 (MIP, 1996). The performance of population crosses observed in this trial followed the heterotic response of crosses between the well known Tux-peno dent and Caribbean flint races of maize (Well-hausen 1978; Goodman, 1985). The populations belonging to the Caribbean heterotic group, namely TZB-SR and Suwan 1-SR, were then crossed to form a broad-based composite known as TZL COMP3 C0. Populations representing the Tuxpeno heterotic group, namely TZPB-SR, POP 43-DMRSR and POP 21-SR, were inter-crossed to form the second broad-based composite referred to as TZL COMP4 C0 (MIP, 1996). Details about the component populations and the steps followed to constitute the two late matur-ing composites have been described in the Maize Improvement Program report (MIP, 1996). A recipro-cal S_2 testcross selection scheme that requires three years to complete a cycle in Nigeria was initiated in 1989 and 1990 to improve performance of TZL COMP3 and TZL COMP4, respectively. In the first se-lection cycle, 1,000 S_2 lines derived from each com-posite were evaluated in two replications for highly heritable traits including standability, resistance to diseases and ear rots, and other agronomic traits at Ikenne in Nigeria where naturally occurring disease pressure is high. Amongst these, 300 S_2 lines with de-sirable agronomic features and good levels of resis-tance to diseases were selected to form testcrosses with the reciprocal composite. Bulk pollen collected from the reciprocal population was used to pollinate the S_2 female rows. The resulting testcrosses were evaluated in replicated trials at two to three locations. The best 40 to 50 S_2 lines of testcrosses identified us-ing a selection index designed to combine high yield potential with acceptable days to anthesis and silk-ing as well as other desirable agronomic traits were inter-mated to form the first selection cycle (C1). Dur-ing subsequent selection cycles, more than 500 S_1 lines were derived from each composite followed by selection of the best 165 to 250 S_1 lines to form full-sib families. The selected 165 to 250 S_1 were planted in paired rows to generate testcrosses, which were evaluated in replicated trials in three to four loca-tions. Again a selection index generated from mean testcross performance averaged over three locations was used to identify the best 26 to 30 S_1 lines from each composite for inter-mating to form the second (C2), third (C3) and fourth (C4) selecton cycles.

The best 26 S_1 lines each selected from the C3 of TZL COMP3 and TZL COMP4 for inter-mating to form the C4 were planted at Saminaka in Nigeria in 2007. More than 10 plants with desirable agronomic traits and good synchrony between pollen shed and silking were self pollinated in each S_1 line. The best 90 S2 lines derived from TZL COMP3 C3 and 67 S_2

lines derived from TZL COMP4 C3 were planted in single rows at Saminaka in 2008 and desirable plants were self pollinated in each line to generate S_3 bulk seeds. In 2009, 41 S_3 lines derived from TZL COMP3 C3 and 38 S_3 lines derived from TZL COMP4 C3 were selected and planted in single rows at Saminaka in Nigeria and uniform plants with desirable agronomic traits were self pollinated in each line to generate 36 S4 bulk seeds for each composite. The 36 S_4 lines representing each of the two composites were planted in paired rows to form 36 testcrosses in 2010.

Field performance evaluation

A trial composed of the 36 testcrosses and duplicate entries of two commercial hybrid that are widely cultivated in Nigeria (Oba Super I and Oba 98) used as checks was planted at Ikenne (3°42´E, 6°54´N, altitude 30 m), Kadawa (8°19´E;12°2´N, altitude 520 m), Saminaka (8°39´E;10°34´N, altitude 760 m), and Zaria (7°21´E;11°7´N, altitude 640 m) in 2010 and 2011 and at Mokwa (9°18´N;5°04´E, altitude 210 m) in 2010. The hybrids were arranged in 5 x 8 alpha lattice design with two replications and were planted in single row plots, 5 m long with 0.75 m spacing between rows and 0.50 m spacing between plants within a row, to attain a population density of 53,000 plants ha^{-1}. Fertilizer and field management practices recommended for optimum maize production were used at each location.

In each plot, days to anthesis and days to silking were recorded as the number of days from planting to when 50% of the plants were shedding pollen and showing emerged silks, respectively. Plant and ear heights were measured in cm as the distance from the base of the plant to the height of the first tassel branch and the node bearing upper ear, respectively. Plant aspect was rated on a scale of 1 to 5, where 1 = excellent overall phenotypic appeal and 5 = poor overall phenotypic appeal. Ear aspect was scored on a 1 to 5 scale, where 1 = clean, uniform and large ears and 5 = rotten, variable and small ears. *Puccinia polysora* rust (southern corn leaf rust) and *Bipolaris maydis* blight (southern corn leaf blight) were scored at Ikenne for two seasons on a scale of 1 to 5, where 1 = slight leaf infection and 5 = severe leaf infection. The total number of plants and ears were counted in each plot at the time of harvest. The number of ears per plant was then calculated as the proportion of the total number of ears at harvest divided by the total number of plants. All ears harvested from each plot were weighed and representative samples of ears were shelled to determine percent moisture. Grain yield adjusted to 15% moisture was computed from ear weight assuming a shelling percentage of 80%.

DNA extraction

Young leaves were collected from five maize seedlings of each S_4 line grown in the field for three weeks. The bulked leaf tissue samples were lyophilized, ground, and DNA was extracted using a modified CTAB protocol of (Saghai-Maroof et al, 1984). The quality of the DNA for genotyping by sequencing (GBS) was ascertained by digesting the DNA with restriction enzyme HindIII. The genomic DNA was then transferred into a 96 well plate, properly sealed with rubber plate covers, and sent to Institute for Genomic Diversity (IGD) of Cornell University (Ithaca, NY for genotyping

Genotyping with SSR

The 57 SSR primers used for genotyping the S4 lines were chosen from the Maize GDB database (http://nucleus.agron.missouri.edu/cgi-bin/ssr_bin.pl) based on their bin locations that cover the ten chromosomes (ch) in the maize genome. The number of the SSR loci ranged from 3-8 per chromosome (Supplemental Table 1). Oligonucleotide primers were purchased from the Integrated DNA Technologies Leuven, Belgium (IDT). PCR reaction conditions, gel electrophoresis and gel visualisation were performed as described by (Senior et al, 1998). Variable annealing temperature, including 65-55°C, 70°C-63°C and 60°C-50°C were used during PCR reactions. The SSR loci amplified were separated on 2% (w/v) superfine agarose gels. The gel was stained with ethidium bromide solution and photographed under UV light attached to a gel documentation system (Bio-Rad, Hercules, CA). Only clear and unambiguous polymorphic bands detected with SSR markers were scored manually as present (1) or absent (0) for all the S4 lines to generate a binary data.

Genotyping with SNP

GBS libraries were prepared and analyzed as described by (Elshire et al, 2011) using the enzyme ApeKI for digestion and creating a library with unique barcodes for each genotype. Raw reads from the sequenced GBS library were called in the GBS analysis pipeline Tassel version 3.0.147, an extension to the Java program TASSEL (Bradbury et al, 2007). The filtered sequences were aligned to the maize reference genome B73 RefGen v1 (Schnable et al, 2009) using the Burrows-Wheeler alignment tool (BWA). This procedure provided 143,415 SNPs covering all the ten chromosomes of the maize genome. Out of these, 2,263 SNP loci having a minimum of 0.05 allele frequency and no missing data, were selected using TASSEL version 4.1.12 and used for analyzing the genetic S4 lines in the current study. Map distribution of the SNP loci on the 10 maize chromosomes (ch) was 355 in ch1, 250 in ch2, 262 in ch3, 224 in ch4, 213 in ch5, 195 in ch6, 221 in ch7, 206 in ch8, 171 in ch9, and 166 in ch10.

Analysis of field data

In the combined analysis of variance for agronomic traits, each location-year combination was considered an environment. The analysis was computed with PROC MIXED procedure in PC-SAS (SAS Institute, 2009) that considered environments, replications and blocks within replications as random effects and test-

Table 1 - Mean squares for selected sources of variation for agronomic traits measured in testcrosses evaluated at nine test environments in Nigeria.

Source	DF	Grain yield	Anthesis	Silking	Plant height	Ear height	Plant aspect	Ear aspect	Southern corn leaf rust	Southern corn leaf blight
Environment (ENV)	8	145345595***	1723.13***	1688.10***	19493***	15138***	1.20*	6.13**	0.4	0.31
Replication (ENV)	9	4876436	39.12**	46.67**	1070.38*	835.83**	0.34	0.53	0.49*	0.51
Block (ENV*REP)	72	2576863***	6.73***	9.35***	281.00***	175.82***	0.23**	0.27**	0.06	0.12*
Testcross	39	16839151***	19.23***	21.69***	1032.46***	779.10***	1.49***	1.79***	0.15	0.51***
ENV*Testcross	312	2352886***	4.06**	4.69**	101.63	92.48	0.22**	0.30***	0.12**	0.13**

*, **, *** Significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$ levels, respectively.

crosses as fixed effects. The significance of the mean squares for the main and interaction effects were tested using the appropriate mean squares, obtained from the Type-3 mixed model analysis (SAS Institute, 2009). To assess consistency of testcross performance across the nine test environments, Kendall's (1962) coefficient of concordance (W) was computed for each trait based on ranks of testcross means recorded in the nine environments. Repeatability values for agronomic traits were estimated using PROC MIXED procedure in PC-SAS (SAS Institute, 2009) as described by (Holland et al, 2003). To separate testcrosses involving pairs of S_4 lines derived from the two composites based on combination of agronomic traits, principal component analysis was computed in SAS (SAS Institute, 2009) using the correlation matrix of mean values of all traits averaged over nine environments, except grain yield. The first two principal component axes (PC1 and PC2) scores were plotted to visualise the separation of the testcrosses from the commercial checks and to determine the correlation between a combination of traits defined by each component axis and grain yield of the testcrosses.

Analysis of marker data

For both the SSR and SNP markers, allele frequency, number of composite specific alleles, gene diversity, polymorphic information content (PIC), and pair-wise Roger's (1972) genetic distance estimates among the 72 S_4 lines were calculated using PowerMarker version 3.25 (Liu and Muse, 2005). The difference between mean genetic distance within and between composites was evaluated using t-test. Principal component analysis (PCA) was calculated based on the Roger's genetic distance estimates from the SSR and SNP markers to determine associations among the 72 S4 lines with PC-SAS (SAS Institute, 2009). Analysis of Molecular Variance (AMOVA) was also computed to assess the variation within or between the two composites using the ARLEQUIN 3.0 software (Schneider et al, 2000). Significance of variance components was tested using a non-parametric procedure based on 1,000 random permutations of individual S_4 lines using the ARLEQUIN software (Schneider et al, 2000). The level of differentiation between the two sets of S_4 lines derived from the two composites was tested by using pair-wise FST distance comparison with 1,000 random permutations of individual lines between reciprocal composites using the ARLEQUIN software (Schneider et al, 2000).

Results

Agronomic performance of testcrosses of S_4 lines

The trial in the present study was evaluated in nine test environments with diverse physical properties and growing conditions that resulted in average mean grain yields varying from 3,740 kg ha^{-1} to 7,908 kg^{-1}. In the combined analyses of variance, environment had significantly affected grain yield and other traits, except southern corn leaf rust and southern corn leaf blight (Table 1). The variance among testcrosses was significant for all traits, except for southern corn leaf rust. The testcrosses x environment interaction mean squares were also significant for all traits, except for plant height and ear height (Table 1). However, the variance for hybrids was 4 to 10 times larger than the corresponding variance for testcrosses x environment interaction for all agronomic traits, except for southern corn leaf rust. Further assessment of consistency of the relative ranking of testcross trait means found significant (P < 0.05 to P <0.001) coefficient of concordance (W) for grain yield and other traits (W = 0.26 to W = 0.76) recorded across the nine test environments. Repeatability estimates for all traits varied from 0.75 to 0.91, except for southern corn leaf rust (0.34), indicating detection of considerable levels of genetic variability across the nine test environments (Supplemental Table 2).

The testcrosses of pairs of S_4 lines derived from the two composites produced grain yields varying from 3,302 kg ha^{-1} to 7,637 kg ha^{-1} whereas the commercial hybrid checks had mean grain yields ranging from 4,718 kg ha^{-1} to 5,043 kg ha^{-1}. Amongst the 36 testcrosses, 22 yielded significantly more than the highest yielding commercial hybrid check (Oba 98). These testcrosses produced 1,065 kg ha^{-1} to 2,594 kg ha^{-1} more grain yields in comparison to Oba 98. The remaining 13 of the 14 testcrosses had grain yields that were not significantly different from the yield of Oba 98 (Supplemental Table 2). The best 22 testcrosses had anthesis and silking days that were similar to or 1 day later than those of the commercial hybrids (Supplemental Table 2). Also, mean plant and ear heights of the best testcrosses were comparable to or greater than those of the commercial hybrids, whereas mean plant aspect, ear aspect and disease scores of these testcrosses were better than those of the commercial hybrids (Supplemental Table 2).

Inbred lines derived from the two composites should impart not only high yield potential but also

Table 2 - Eigenvectors of the first two principal component axes (PC1 and PC2) computed from correlation matrix of testcross trait means recorded in 9 environments in Nigeria.

	PC1	PC2
Anthesis (days)	-0.26**	0.59****
Silking (days)	-0.18*	0.58****
Plant height (cm)	-0.02	0.23
Ear height (cm)	-0.15	0.35**
Plant aspect (1-5)	0.49****	0.28*
Ear aspect (1-5)	0.47****	0.15
Southern corn leaf rust (1-5)	0.44****	0.20
Southern corn leaf blight (1-5)	0.46****	0.03
Correlation with grain yield	-0.46**	-0.23
Variance	0.39	0.23

*, **, ***, **** Significant at $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$ levels, respectively.

desirable agronomic traits in hybrids to be successfully used as parents. Principal component analysis was then computed to integrate the major agronomic traits into unrelated component axes in the current study. The first two principal component axes (PC1 and PC2) explained 60% of the total variation in agronomic traits among hybrids (Table 2). Large PC1 axis scores were significantly associated with early anthesis and silking days, poor plant and ear aspect scores, and increased susceptibility to southern corn leaf rust and southern corn leaf blight. The most important traits that contributed significantly to PC2 with positive values were days to anthesis and silking, ear height, and plant aspect (Table 2). Correlation analyses found significant and negative association between grain yield and PC1 axis scores but not between grain yield and PC2 axis scores (Table 2). The scatter plot for grain yields and PC1 axis scores showed a clear separation of the testcrosses of pairs of S_4 lines from the commercial hybrids (Figure 1). Also, the testcrosses of S_4 lines were more dispersed than the commercial hybrids, showing greater genetic diversity in grain yield and other traits. We found several testcrosses between pairs of S_4 lines that combined higher grain yields with desirable agronomic traits including resistance to diseases in comparison to the commercial hybrid checks (Figure 1; Supplemental Table 2).

Marker-based genetic diversity of the S_4 Lines

The 72 S_4 lines derived from the two composites were genotyped with SSR and SNP markers. The 57 SSR primers detected a total of 212 alleles whereas the SNPs detected a total of 4,526 alleles across all the lines. As compared to the biallelic SNPs, the SSR primers detected 2 to 6 alleles at each locus with an average of 3.7. The proportion of minor alleles occurring at frequencies of less than 0.25 was 49% for SSRs and 43% for SNPs. Only 16% of the alleles at the SSR loci occurred at frequencies exceeding 0.50 whereas 50% of the alleles at the SNP loci were found at frequencies of more than 0.50 across the S_4 lines. The number of SSR alleles found exclu-

sively in a single composite was 30 for TZL COMP3 and 17 for TZL COMP4, whereas composite-specific SNP alleles were 202 for TZL COMP3 and 10 for TZL COMP4. The composite-specific alleles were found at frequencies of 0.75 or less for SSRs and at frequencies of 0.42 or less for SNPs. The PIC values varied from 0.14 to 0.82 with an average of 0.60 for the SSRs and from 0.10 to 0.38 with an average of 0.20 for SNPs. The average gene diversity was 0.65 for SSRs and 0.23 for SNPs.

The distribution of the genetic diversity in the S4 lines was determined using AMOVA of the SSR and SNP data (Table 3). The results revealed that more than 80% of the total genetic variance resided within composites with the remaining 14% and 7% of the total variation in SSR and SNP data, respectively, accounting for differences between the two composites (Table 3). The observed genetic differentiation between the two composites using AMOVA was consistent with the results of Wright's fixation index (F_{ST}) calculated from allele frequencies for SSRs (F_{ST} = 0.1428, $P < 0.001$) and SNPs (F_{ST} = 0.0783, $P < 0.001$). To examine the genetic diversity patterns, we computed genetic distances (GD) for pairs of S_4 lines derived from each composite as well as those derived from the two composites using SSR and SNP data (Table 4). Results revealed a broad range of GD estimates between lines derived from each composite as well as those derived from the two composites. The average GD estimates for pairs of lines calculated using SSRs was three times greater than the average GD estimates for pairs of lines computed using SNPs. The average GD estimates for pairs of lines from the two composites was significantly larger than that from pairs of lines derived from each composite tor the two classes of markers. The average GD estimates for TZL COMP3 was significantly larger than that of TZL COMP4 for SNPs but not for SSRs. Simple correlation coefficient between GD estimates based on SSRs and SNPs was 0.41 ($P < 0.001$) for
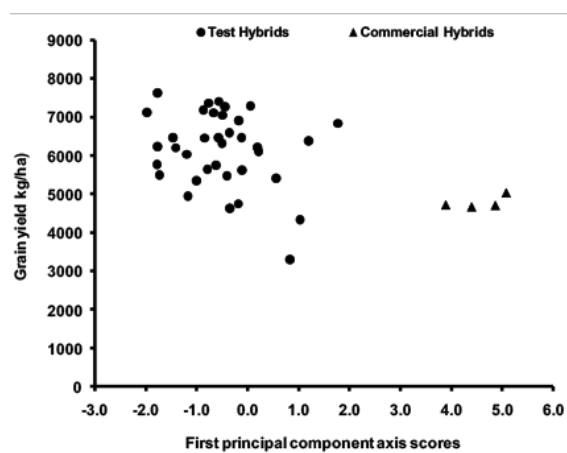

Figure 1 - Principal component analysis of testcross trait means recorded in 9 environments in Nigeria in 2010 and 2011.

Table 3 - Analysis of molecular variance (AMOVA) and Wright's fixation index (F_{ST}) for 72 S_4 lines derived from two reciprocal composites based on 212 SSR and 4562 SNP alleles.

Source of variation	df	Sum of square	Variance component	Percentage of variation	F_{ST}
			SSR		
Between composites	1	226.95	5.40	14.28	0.1428****
Within composites	70	2269.47	32.44	85.72	
			SNP		
Between composites	1	1432.06	29.97	7.83	0. 0783****
Within composites	70	24712.33	353.03	92.17	

**** Significant at P < 0.0001.

all pairs of S_4 lines and varied from 0.56 (P < 0.001) for pairs of lines from TZL COMP3 to 0.35 (P < 0.001) for pairs of lines derived from TZL COMP4. Principal component analysis was computed to further assess the genetic diversity pattern of the S_4 lines. The first (PC1) and second (PC2) principal component axis accounted for 24% and 10% of the total variance in SSRs and 41% and 8% of the total variance in SNPs, respectively. As shown in Figure 2, the two axes separated the S4 lines into two groups along composite lines, except for a few lines derived from one composite overlapping with lines derived from another composite for SNPs.

Discussion

The development of hybrids with enhanced expression of heterosis depends on the genetic backgroudnd of the source population and the effectiveness of a breeding scheme used to identify and use divergent inbred lines with good combining ability. In the present study, testcrosses of pairs of S_4 lines derived from selfed progenies selected for inter-mating to form C4 of TZL COMP3 and TZL COMP4 were evaluated in nine diverse environments. All testcrosses except one produced grain yields that were competitive to or greater than the highest yielding commercial hybrid. The best testcrosses amongst these yielded 21% to 51% more than the highest yielding commercial hybrid. Other studies also showed that hybrids formed from pairs of inbred lines derived from advanced selection cycles of reciprocal populations produced higher grain yields than the commercial hybrids (Jenweerawat et al, 2010; Souza Jr

et al, 2010). The observed superior agronomic performance of several testcrosses in the present study provide evidence that the S_4 lines derived from the two composites contained favourable complementary alleles with dominance effects at different loci, consistent with results in other studies (Keeratinijakal and Lamkey 1993; Stojsin and Kannenberg 1994; Landi and Frascaroli, 1995). It appears that the selection of the best selfed progenies for inter-mating based on high grain yields and other desirable traits of their testcross during the three selection cycles had increased the importance of specific combining ability effects over general combining ability effects in the two composites, which was in agreement with the results reported in other studies (Doerksen et al, 2003; Santos et al, 2007; Souza Jr et al, 2010). Keeratinijakal and Lamkey (1993) found partial to complete dominance effects to be more important than additive effects for grin yield of inter-population crosses between two reciprocal populations. Other studies also detected directional dominance affecting grain yield in the inter-population crosses (Moll and Hanson 1984; Hanson and Moll, 1986; Eyherabide and Hallauer, 1991). Moreover, the testcrosses exhibited consistent ranking for grain yield across the diverse test environments, indicating an increase in the frequencies of favourable alleles for adaptation to diverse test environments (Ordas et al, 2012) possibly because of selecting progenies with superior agronomic performance in different test locations with diverse climatic conditions for inter-mating during the various cycles of RRS.

Changes in other traits in addition to grain yield would determine the usefulness of inbred lines as

Table 4 - Minimum, maximum and mean genetic distance (GD) estimates for S_4 lines derived from two composites computed based on SSR and SNP data.

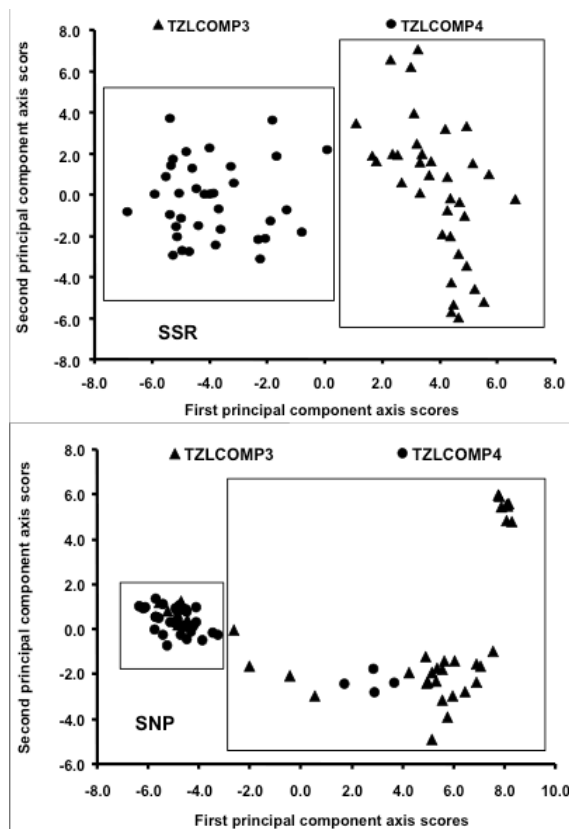| Line combination	Minimum	Maximum	Mean	Std Error
		SSR		
TZL COMP3	0.09	0.84	0.61	0.005
TZL COMP4	0.49	0.88	0.70	0.002
TZL COMP3xTZL COMP4	0.26	0.79	0.61	0.003
All	0.09	0.88	0.65	0.002
		SNP		
TZL COMP3	0.06	0.26	0.21	0.001
TZL COMP4	0.08	0.26	0.22	0.001
TZL COMP3xTZL COMP4	0.09	0.24	0.19	0.001
All	0.06	0.26	0.21	0.001

Figure 2 - Scatter plot of PC1 (24%) and PC2 (10%) for SSR and PC1 (41%) and PC2 (8%) for SNP markers.

potential parents of acceptable hybrids to farmers. In the present study, high grain yields of testcrosses were associated with improvements in overall plant and ear appearance as well as increase in resistance to foliar diseases and ears per plant but were accompanied by a significant delay in days to anthesis and silking. Even though the observed time to anthesis and silking of testcrosses of the S_4 lines fell within the range acceptable for production conditions in WCA, these traits should be monitored in subsequent selection cycles to circumvent changes in undesirable direction. The S_4 lines that formed hybrids with high grain yields and other desirable agronomic traits in the present study can then be subjected to further inbreeding and selection to generate homozygous parental lines for use to develop hybrids with superior agronomic performance and adaptation to diverse production environments. Further improvements of the two composites are expected to increase the probability of developing divergent new inbred lines with good combining ability whose single-cross hybrids outperform the best commercial hybrids adapted to the savannas in WCA. Carena (2005) reported that inbred lines developed from broad-based populations were endowed with superior combining ability with inbred lines selected from more than one heterotic group, suggesting that the inbred lines derived from the two composites may combine well

with other heterotic groups to optimize expression of heterosis in hybrids in WCA. These lines may also be used as parents to make crosses with elite lines belonging to well defined heterotic groups to develop new maize inbred lines containing novel alleles and multiple desirable traits.

The selection of prospective commercial hybrids depends not only on superior agronomic performance but also on the seed production potential of the parents to facilitate hybrid seed production at low cost. Further improvements of the two composites using RRS may constantly supply new early generation lines selected for both per se performance and superior combining ability into the inbred line development process (Betran and Hallauer 1996; Hallauer 2010; Ordas et al, 2012). These lines can be subjected to further inbreeding and selection to develop productive inbred lines with superior agronomic performance in hybrid combinations. There are good examples in which inbred lines derived from populations improved using recurrent selection such as B14, B37, and B73 that have been used extensively as female parents in commercial hybrid development programs (Tracy and Chandler, 2004). The advanced cycles of the two composites have then the potential to be sources of outstanding and unique inbred lines (Carena, 2005) that can be used as parents of pedigree populations and hybrids for emerging and established seed companies in WCA.

TZL COMP3 and TZL COMP4 are composites formed by inter-crossing broad-based populations representing two heterotic pools, which were chosen based on the results of diallel analysis of late maturing maize populations (MIP, 1996). The SSR and SNP markers detected a large number of common and some composite-specific alleles occurring at varying frequencies in the S_4 lines derived from the two composites, resulting in a broad range of GD estimates found within the two composites. However, SSRs detected higher levels of GD estimates among the S_4 lines within and between the two composites than the SNP markers, consistent with the findings in other studies (Hambline et al, 2007; Jones et al, 2007; Ingelandt et al, 2010; Frascaroli et al, 2013). In spite of the differences between SSRs and SNPs, the same trends were observed for GD estimates and the number of composite-specific alleles detected with the two marker types. Pairs of S_4 lines derived from the two composites had larger GD estimates than those derived from each composite for both SSRs and SNPs, indicating the potential of the two reciprocal composites as sources of divergent lines for use to develop hybrids. The inbred lines derived from the two composites carrying composite-specific alleles can also be important donors of novel genetic variation for pedigree breeding to enhance allelic diversity in adapted germplasm.

In our analysis of the genetic structure of the S_4 lines using SSRs and SNPs, the largest percentage

of the total variation at the molecular level was found among lines within the two composites, which is in agreement with results from another study in maize (Heinze et al, 2005). The two types of markers separated the S_4 lines along the two composites, with only a few lines from one composite clustering with lines from another composite when SNPs were used. SSRs were better than SNPs in separating the S_4 lines into distinct groups possibly due to the higher mutational rates of the SSRs that provide greater opportunities for genetic drift to create detectable changes in allelic frequencies between reciprocal composites and the accumulation of campsite-specific alleles (Haasl and Payseur, 2010). The difference in genetic structure of the reciprocal composites observed at the molecular level may be caused by the effects of genetic drift, inbreeding, selection, and hitchhiking of alleles linked with selected alleles (Labate et al, 1999; Pinto et al, 2003; Falke et al, 2007; Rommay et al, 2012).

In summary, pairs of S_4 lines derived from selfed progenies selected to form the C4 of the reciprocal composites generated several testcrosses with high grain yields and other desirable agronomic traits. It appears that the reciprocal composites carried different sets of alleles, facilitating the heterozygous condition at loci in inter-composite hybrids, contributing to the superior performance of several testcrosses in the present study. SSRs and SNPs detected a broad range of genetic diversity among the S_4 lines, which was structured along the two composites. Such information may allow potential users to select diverse lines from each composite for pedigree breeding and hybrid development. The inbred lines derived from the two composites may likely have a wealth of useful alleles for agronomic and defensive traits not represented in elite inbred lines and genetic material that are presently exploited by breeders in the national programs and private seed companies in WCA. These lines may then be used not only as new and unrelated parents to existing lines for developing hybrids but also as sources of diverse alleles for broadening the genetic base of adapted germplasm to sustain genetic gain in productivity of hybrids in this region.

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