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Grouping of tropical mid-altitude maize inbred lines on the basis of yield data and molecular markers

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Abstract The classification of maize inbred lines into heterotic groups is an important undertaking in hybrid breeding. The objectives of our research were to: (1) separate selected tropical mid-altitude maize inbred lines into heterotic groups based on grain yield data; (2) assess the genetic relationships among these inbred lines using amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers; (3) examine the consistency between yield-based and marker-based groupings of the inbred lines. Thirty-eight tropical mid-altitude maize inbred lines were crossed to two inbred line testers representing the flint and dent heterotic pattern, respectively. The resulting testcrosses were evaluated in a trial at three locations for 2 years. Significant general combining ability (GCA) and specific combining ability (SCA) effects for grain yield were detected among the inbred lines. The tester inbred lines classified 23 of the 38 tested inbred lines into two heterotic groups based on SCA effects and testcross mean grain yields. This grouping was not related to endosperm type of the inbred lines. The outstanding performance of testcrosses of the remaining 15 inbred lines indicates the presence of significant genetic diversity that may allow the assignment of the lines into more than two heterotic groups. Diversity analysis of the 40 maize inbred lines using AFLP and SSR markers found high levels of genetic diversity among these lines and subdivided them into two main groups with subdivision into sub-groups consistent with breeding history, origin and parentage of the lines. However, heterotic groups formed using yield-based combining ability were different from the groups established on the basis of molecular markers. Considering the diversity of the genetic backgrounds of the mid-altitude inbred lines, the marker-based grouping may serve as the basis to design and carry out combining ability studies in the field to establish clearly defined heterotic groups with a greater genetic similarity within groups.

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Introduction

The mid-altitude agroecolgical zone falls between 800 m.a.s.l. and 1,800 m.a.s.l. and encompasses about 40% of the land area in sub-Saharan Africa, mainly in eastern and southern Africa. Maize is an important crop in the mid-altitude zone of eastern, central, southern and western Africa, and the ecological conditions found in this zone, including cool temperatures that enable a long developmental cycle, adequate rainfall in most areas and some fertile volcanic soils (CID 1993), are favourable for a high yield potential of this crop. Since cool temperatures also inhibit the breakdown of soil organic matter, the soils in this zone often have a higher organic matter content and a greater cation exchange capacity than soils of low altitudes (CID 1993). The high yield potential of the mid-altitudes provides an opportunity to exploit the greater yield advantages that hybrids can provide. Various diseases and pests, such as *Exserohilum turcicum*, *Puccinia sorghi*, *Diplodia macroformina*, *Cercospora zeamaydis* and *Busseola fusca*, are found in this zone for which lowland germplasm is often susceptible (CID 1993). As a result of this, different germplasm complexes are needed.

The International Institute of Tropical Agriculture (IITA) initiated inbred line development and hybrid research for the tropical mid-altitude zone in Nigeria in 1980. Inbred lines extracted from crosses between maize streak virus-resistant populations and other diverse sources of germplasm (IITA 1984) were supplied as source germplasm to initiate a joint hybrid project between IITA

and the Cameroon Institute of Agronomic Research (IRA) in Cameroon in 1984 (Kim et al. 1985).

The classification of elite germplasm and inbred lines into heterotic groups is an important undertaking in any breeding programme for hybrid maize (Hallauer et al. 1988). Unlike the situation in temperate maize germplasm, limited information is available on heterotic groups among maize inbred lines and populations adapted to the tropical mid-altitudes (Vasal et al.1992a; Everett et al. 1995). Although the mid-altitude inbred lines developed in Nigeria and Cameroon have been tested in hybrid combinations with selected lines, a systematic study aimed at classifying these lines into heterotic groups has not been reported. Such information would be useful in the development of inbred lines within groups and the generation and evaluation of maize hybrids and open-pollinated synthetic varieties between groups.

Melchinger (1999) proposed that, when a large number of inbred lines is available and proven testers exist, the relative performance of the lines in testcrosses with proven testers can be used as a main criterion for grouping of the lines. Vasal et al. (1992a, 1992b) used this approach to evaluate the performance of testcrosses of 92 tropical and 88 subtropical maize inbred lines with two dent and two flint tester lines. The lines exhibiting contrasting specific combining ability (SCA) effects with two of the four testers were placed into separate heterotic groups. Considering the mixed genetic composition and the broad genetic base of the source populations for the tropical mid-altitude inbred lines, it may be difficult to classify these lines into distinct heterotic groups based only on the results of combining ability studies. Therefore, the combined use of molecular markers that allow direct comparison of the similarity of inbred lines at the DNA level with testcross evaluation may facilitate the separation of these lines into well-defined heterotic groups.

Molecular markers such as amplified fragment length polymorphisms (AFLP) and simple sequence repeats (SSR) have been used to assess genetic relationships among maize inbred lines and to classify lines into heterotic groups (Ajmone-Marsan et al. 1998; Pejic et al. 1998; Senior et al. 1998; Lűbberstedt et al. 2000; Lu and Bernardo 2001; Enoki et al. 2002). SSR and AFLP diversities have been strongly correlated with restriction fragment length polymorphism (RFLP) diversity and show good agreement with pedigree data in temperate maize (Smith et al. 1997; Lűbberstedt et al. 2000). A comparative study of AFLP and SSR markers in maize generally showed good agreement between the genetic patterns revealed by the two molecular markers (Pejic et al. 1998).

The objectives of the investigation reported here were (1) to separate selected tropical mid-altitude maize inbred lines into heterotic groups based on grain yield of testcrosses; (2) to use a combination of AFLP and SSR markers to assess the genetic relationships among these inbred lines; (3) to examine the consistency between yield-based and marker-based groupings of the tropical mid-altitude inbred lines.

Materials and methods

Genetic materials

Forty tropical mid-altitude maize inbred lines at the S_9–S_{10} generation developed at the Jos Plateau in Nigeria and at the Adamaoua Plateau in Cameroon were used for this study. As shown in Table 1, these inbred lines were derived from a maize streak virus-resistant mid-altitude population, TZMSR, crosses between lowland maize streak virus-resistant populations and mid-altitude germplasm from eastern and southern Africa as well as other germplasm sources from CIMMYT and the temperate zone (IITA 1984). The major source of the inbred lines, TZMSR, was a mid-altitude adapted population formed by crossing the best varieties and hybrids from eastern, southern and central Africa to a maize streak virus-resistant germplasm (IITA 1984). The streak-resistant

Table 1 List of maize inbred lines included in the study along with their parentage, grain texture and cluster number to which they were assigned based on molecular markers

Inbred	Parentage	Origin	Grain texture[a]
89183	TZMSR	Cameroon	F
89207	TZMSR	Cameroon	F
89365	S85×C70	Cameroon	FD
90156	SynA1×87004	Cameroon	F
90183	SynA1×87014	Cameroon	FD
90219	SynA1×87014	Cameroon	FD
90267	SynA1×87036	Cameroon	F
90323	SynB1×87036	Cameroon	FD
TZMI103	TZMSR-W	Nigeria	F
TZMI210	Cameroon line 1423-26	Nigeria	F
TZMI214	F88B 114-68-1-1-1	Nigeria	F
TZMI303	UCA SR S4 7-9-1-2-1	Nigeria	D
TZMI604	Population 32×SynA1. 146	Nigeria	F
88069	ZS206×TZMSR	Cameroon	FD
89246	TZMSR	Cameroon	DF
89291	(ZS206×TZMSR)×ZS206	Cameroon	D
89302	(H625×Z10)×H625	Cameroon	F
TZMI104	TZMSR-W	Nigeria	F
TZMI201	Zambia×TZSR	Nigeria	F
TZMI205	Kit2×TZMSR×Kit2	Nigeria	FD
TZMI209	H625×Z10	Nigeria	FD
TZMI211	Cameroon hybrid self. S4-9	Nigeria	FD
89248	TZMSR	Cameroon	FD
89260	H625×Z10	Cameroon	F
90113	SynA1×87004	Cameroon	F
TZMI107	TZMSR IPTT S3 184	Nigeria	FD
TZMI203	Nat'l var. × TZSR	Nigeria	F
TZMI206	TZMSR×East African hybrid	Nigeria	F
TZMI212	F88B 54-1-1	Nigeria	F
TZMI606	TGS-512	Nigeria	FD
87014	TZMSR	Cameroon	FD
87036	TZMSR	Cameroon	D
89311	M122×N103	Cameroon	F
TZMI204	Nat'l var. × TZSR	Nigeria	D
TZMI208	SYNA1×87014	Nigeria	FD
TZMI305	TZMSR×Kasai BC_5 8-1-1	Nigeria	F
TZMI501	Com. hybrid × TZSR	Nigeria	D
TZMI602	La Posta S7-25	Nigeria	D
TZMI102	TZMSR-W	Nigeria	F
TZMI407	Nat'l var. × TZSR	Nigeria	D

[a] Texture: F, Flint; D, Dent; DF, Semi-dent; FD, Semi-flint

lowland population, TZSR, used as a parent was formed by intercrossing TZB, TZPB, POP 21 and POP 22. The inbred lines exhibited various levels of resistance to the prevailing major diseases in the mid-altitude zone and appeared as parents of at least one high-yielding hybrid in the different testcross trials in Nigeria and Cameroon (Everett et al.; 1994a, 1994b; Kim et al. unpublished). Each of the 38 inbred lines was crossed to two tester lines, TZMI102 and TZMI407, representing each side of the flint and dent heterotic pattern, respectively, in the 1998/1999 dry seasons to generate 76 testcrosses for evaluation in the 1999 and 2000 main cropping seasons.

Field trials

A trial consisting of the 76 testcrosses, a hybrid between the two testers and four experimental hybrids was evaluated at Saminaka (10°40′N, 8°77′E; altitude: 730 m.a.s.l.), Tenti (9°48′N, 8° 48′E; altitude: 1,350 m.a.s.l.) and Vom (9°40′N, 8°50′E; altitude: 1,300 m.a.s.l.) in 1999 and 2000. Saminaka is a transitional zone between the lowland and the mid-altitude and exhibits the disease complexes of the mid-altitude. The hybrids were arranged in a 9×9 simple lattice design and were planted in single-row plots, 5 m long with 0.75 m between rows and 0.25 m between plants within a row. 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 had shed pollen (anthesis) and silks emerged (silking), respectively. Plant and ear heights were measured (in centimeters) as the distance from the base of the plant to the height of the first tassel branch and the node bearing the 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, large and well-filled ears and 5 = rotten, variable, small and partially filled ears. Grey leaf spot (*Cercospora zea-maydis*) was scored at Tenti and Vom on a scale of 1 to 5, where 1 = slight leaf infection and 5 = severe leaf infection. All ears harvested from each plot were weighed, and representative samples of ears were shelled to determine percentage moisture. Grain yield adjusted to 15% moisture was computed from ear weight and grain moisture assuming a shelling percentage of 80% based on the following formula: grain yield (kg/ha) = [ear weight (kg)/area (m^2)] × [(100-moisture)/85] × (10,000×0.80).

Laboratory analyses

Fifteen to twenty 10-day-old seedlings of each inbred line (Table 1) were harvested, frozen and ground into fine powder. The DNA was extracted using the CTAB procedure (Saghai Maroof et al. 1984). Thirty-three SSR primers (bnlg1012, bnlg1035, bnlg105, bnlg1108, bnlg1185, bnlg127, bnlg1917, bnlg2162, nlg2328, dupssr10, dupssr11, dupssr17, dupssr19, dupssr23, macE01C02, mag1F03, mmc0041, mmc0081, mmc0121, phi034, phi037, phi042, phi064, phi087, phi102228, phi126, umc1016, umc1024, umc1029, umc1154, umc1305, umc1355) were purchased from Research Genetics (Huntsville, Ala.). PCR analyses were performed in 15 μl of reaction mixture containing 50 ng genomic DNA template, 10 m*M* Tris-HCl (pH 8.3), 50 m*M* KCl, 2.0 m*M* MgCl2, 0.1% Triton X-100, 0.2 μM of each dNTP, 0.2 μM of each oligonucleotide primer and 1 U *Taq* DNA polymerase. The amplification was carried out in a 96-well DNA Thermal Cycler (MJ Research, Watertown, Mass.) using a "touchdown" programme. The thermocycling profile included an initial denaturation of 94°C for 5 min, followed by one cycle of denaturing at 94°C for 45 s, annealing at 65°C for 60 s and extension at 72°C for 60 s. The annealing temperature was decreased from 65°C to 55°C with a 10°C drop per cycle. The reactions were subsequently subjected to 25 additional cycles after reaching the final annealing temperature. This was followed by a final extension at 72°C for 7 min. Amplified products were mixed with 5 μl of loading dye (60% sucrose, 1 m*M* cresol red dye) and resolved on 2% MetaPhor agarose gels (FMC-Bioproducts, Rockland, Me.). The gels were run at 100 V for 6 h in 1× TBE buffer (89 m*M* Tris-Borate plus 2 m*M* EDTA, pH 8.5). The gels were stained with 0.1% ethidium bromide for 20 min, visualized over a transilluminator and photographed under UV light. Only clear polymorphic SSR bands of different sizes were scored manually in binary formulas of 1 or 0 for their presence or absence, respectively, in each line.

The AFLP analysis was performed following the standard procedure described by Vos et al. (1995). Genomic DNA (0.3 μg) of each line was digested with a pair of restriction enzymes (*Eco*RI and *Mse*I) and ligated to double-stranded adapters. The ligate was preamplified with nonselective primers, and selective amplification was carried out using pairs of selective primers with *Eco*RI/*Mse*I extensions (E40/M49, E32/M49, E35/M62, E37/M50, E33/M62, E35/M50, E37/M47, E38/M60, E32/M62, E33/M61 and E41/M59). The products were separated on polyacrylamide gel and were silver-stained using the manufacturer's instructions (Promega, Madison, Wis.). Only polymorphic bands with a strong intensity were scored manually in a binary form as 1 or 0 for their presence or absence, respectively, in each line.

Statistical analysis

For the field trials, each location-year combination was considered to be a test environment, and analyses of variance were computed for each location-year combination to generate entry means adjusted for block effects according to the lattice design (Cochran and Cox 1960). The pooled error mean square was obtained by dividing the sum of the error sums of square from all location-year combinations ANOVA with the corresponding sum of the error degrees of freedom. The adjusted means were used to conduct a combined analysis of variance. All analyses were performed with PROC GLM in SAS (SAS Institute 1997) using a RANDOM statement with the TEST option. The mean square due to environment × line × tester was tested using the pooled error mean squares. Line × tester analysis was calculated using the adjusted means after the check entries were omitted based on the method described by Kempthorn (1957). General combining ability (GCA) and specific combining ability effects for grain yield and other traits were calculated based on the line × tester model. Correlation coefficients were calculated between combining ability, effects of grain yield and other traits (SAS Institute 1997).

The genetic similarity (GS) between pairs of lines was computed from the 250 AFLP and 101 SSR fragments based on Dice's coefficient (1945) using a SAS macro (Mumm and Dudley, 1995). Genetic distance (GD) estimates were computed from similarity estimates as $GD_{ij}=1-GS_{ij}$. The average genetic distances between pairs of inbred lines bred in each country and between those bred in the two countries were obtained by the MEANS procedure in SAS (SAS Institute 1997). The AFLP-based GD matrix was compared with the SSR-based GD matrix using the MAXCOMP routine of NTSYS-PC (Rolf 1998), and the goodness of fit of the two matrices was assessed using Mantel's test (1967). A neighbour-joining method was used to cluster the inbred lines based on their GD estimates (Rolf 1998). Additionally, principal component analyses (PCO) were performed based on the genetic distance matrices using PC SAS (SAS Institute 1997). Correlations between GD estimates and SCA effects as well as between mean grain yields and GD estimates of the 38 inbred lines in combination with each tester were calculated using PROC CORR of SAS (SAS Institute 1997).

Results

Testcross performance and grouping of lines based on combining ability

In the combined analyses of variance, the environmental effects were significant ($P<0.05$) for all traits except grey leaf spot and plant aspect (Table 2). The difference in GCA effects among mid-altitude inbred lines was highly significant ($P<0.001$) for all traits, whereas the GCA effects between testers was significant ($P<0.05$) for days to silking, plant height, ear aspect, husk cover and grain yield. The SCA effect (line × tester) was significant for all traits except days to silking and ear height. The line × environment and tester × environment interactions were significant for most of the traits (Table 2). On the other hand, the line × tester × environment interactions were not significant for plant height, husk cover, ear rot, grey leaf spot and grain yield (Table 2).

Mean values and estimates of GCA and SCA effects for grain yield of the lines are presented in Table 3. The average grain yield of the 38 inbred lines crossed to TZMI102 in this trial was 443 kg/ha less than the average TZMI407 testcross yield. Mean grain yield varied from 5,320 kg/ha to 8,400 kg/ha for testcrosses of TZMI102 and from 4,787 kg/ha to 8,485 kg/ha for TZMI407 testcrosses. Twenty TZMI102 and 32 TZMI407 testcrosses had a significantly higher grain yield ($P<0.05$) than the hybrid between the two testers, TZMI102×TZMI407. The increased in yield of these hybrids over that of TZMI102×TZMI407 varied from 13% to 41%. Of the remaining 24 testcrosses, only one yielded significantly less than TZMI102×TZMI407. Some of the high-yielding testcrosses were found to be as good as or better than TZMI102×TZMI407 for other agronomic traits (data not shown).

Only ten inbred lines bred in Cameroon and seven inbred lines bred in Nigeria had positive GCA effects for grain yield (Table 3). Among these, four inbred lines from the former and three inbred lines from the latter had significantly positive GCA effects for grain yield. The testers exhibited contrasting combining ability for grain yield, with TZMI102 having negative and TZMI407 having positive GCA effects (Table 3). The GCA effects for grain yield were negatively correlated with GCA effects for days to silking (r=-0.43, $P<0.0001$), plant aspect (r=-0.60, $P<0.0001$), ear aspect (r=-0.64, $P<0.0001$) and grey leaf spot (r=-0.41, $P<0.001$) and positively correlated with plant height (r=0.41, $P<0.001$). Among the inbred lines with positive SCA effects for grain yield, only four lines in combination with each tester had significant SCA effects (Table 3).

We used the combining ability effects and mean grain yields of the tropical mid-altitude maize inbred lines in combination with the flint and dent tester as the basis to classify the lines into heterotic groups. Ten inbred lines showing positive SCA effects with TZMI102 but having negative SCA effects with TZMI407 and with testcross mean yields equal to or greater than the mean yield of TZMI102×TZMI407 were placed into the TZMI102 heterotic group (Table 3). In addition, 13 inbred lines exhibiting positive SCA effects with TZMI407 but having negative SCA effect with TZMI102 and with testcross mean yields equal to or greater than the mean yield of TZMI102×TZMI407 were placed into the TZMI407 heterotic group (Table 3). It is interesting to note that four of the ten testcrosses involving lines assigned to the TZMI102 heterotic group produced slightly lower yields than the corresponding testcrosses of the same lines with TZMI407. On the other hand, 15 lines having positive GCA effects and with significantly ($P<0.05$) higher testcross mean yields than the mean yield of TZMI102×TZMI407 were assigned to both the TZMI102 and TZMI407 heterotic groups (Table 3)

Assessment of genetic diversity and grouping of lines using molecular markers

The 40 mid-altitude maize inbred lines, two of which were used as testers, were surveyed with AFLP and SSR markers. The total number of polymorphic bands detected across all inbred lines was 250 for the 11 AFLP primer combinations and 101 for the 33 SSR primers. The number of polymorphic bands for AFLP primers varied from 15 to 38, with an average of 23, and that for SSR

Table 2 Sums of squares of selected sources of variation, expressed as percentages of the corrected total sums of squares, from the combined analyses of variance for mid-altitude inbred lines evaluated in crosses with two testers at three locations in Nigeria in 1999 and 2000

Source	Days to silk[a]	Plant height[a]	Ear height[a]	Plant aspect[a]	Ear aspect[a]	Husk cover[a]	Grey leaf spot[a]	Grain yield[a]
Environment (ENV)	93.5***	34.6***	27.4***	24.7	15.1*	47.7***	12.5	53.9***
Line	2.2***	22.2***	12.6***	5.9**	20.4***	12.2***	18.2***	7.6***
Tester	0.1*	2.0*	0.4	4.8	6.3*	0.5*	20.7	1.2*
Line × tester	0.2	2.9*	4.4	4.6**	6.5***	3.0*	8.6***	3.2***
Line × ENV	1.4***	12.0**	16.1	16.7	18.1***	9.8	11.3**	10.4**
Tester × ENV	0.0	0.7**	0.4	5.4***	2.9***	0.3	6.4***	0.5*
Line × tester ENV	0.9*	7.6	13.2**	13.3**	10.5*	8.0	7.0	6.7

*,**, ***Mean squares significant at $P<0.05$, $P<0.01$ and $P<0.001$, respectively
[a]Expressed as the percentage of corrected total sums of squares

Table 3 Mean grain yield, general and specific combining ability (GCA and SCA) effects and yield- and marker-based grouping of 38 tropical mid-altitude maize inbred lines

Inbred	Mean grain yield with:		GCA effects (kg/ha)	SCA effects with TZMI102[a]	Yield-based heterotic groups	SSR-based groups	AFLP-based groups
	TZMI102	TZMI407					
89248	7,246	6,425	−158	638	102	2	1
90113	6,841	7,031	−58	132	102	2	1
90219	6,696	6,820	−236	165	102	1	1
90323	6,521	6,714	−376	131	102	1	1
TZMI103	6,822	7,099	−34	89	102	1	1
TZMI201	6,533	6,085	−685	451	102	1	1
TZMI204	6,466	4,787	−1,368	1,067	102	2	2
TZMI303	7,418	6,462	−54	705	102	1	1
TZMI305	7,062	6,924	−1	296	102	2	1
TZMI501	6,229	6,219	−770	232	102	2	2
89183	6,542	8,006	280	−505	407	1	1
89311	5,969	7,433	−293	−505	407	2	1
89365	6,212	7,270	−253	−302	407	1	1
90156	6,181	7,288	−260	−326	407	1	1
90267	6,412	7,286	−145	−209	407	1	1
TZMI203	6,241	6,917	−415	−111	407	2	1
TZMI206	5,946	7,351	−346	−475	407	2	2
TZMI208	5,859	7,019	−555	−353	407	2	1
TZMI209	6,143	6,852	−496	−127	407	1	1
TZMI212	5,320	6,816	−926	−521	407	2	2
TZMI602	6,410	6,999	−289	−67	407	2	1
TZMI604	6,499	7,643	77	−345	407	1	1
TZMI606	5,373	7,004	−805	−588	407	2	2
87014	7,381	8,059	726	−112	102, 407	2	1
87036	7,157	8,294	732	−341	102, 407	2	2
88069	7,758	7,615	692	299	102, 407	1	1
89207	7,202	7,195	205	231	102, 407	1	1
89246	6,845	7,309	83	−5	102, 407	1	1
89260	6,925	7,348	143	16	102, 407	2	1
89291	7,088	7,876	489	−167	102, 407	1	1
89302	7,116	7,764	446	−97	102, 407	1	1
90183	8,122	7,213	674	682	102, 407	1	1
TZMI104	7,245	7,264	261	218	102, 407	1	1
TZMI107	7,498	7,794	652	79	102, 407	2	1
TZMI205	8,400	8,458	1,435	198	102, 407	1	1
TZMI210	6,993	8,041	523	−297	102, 407	1	1
TZMI211	7,664	8,485	1,081	−183	102, 407	1	1
TZMI214	6,792	7,240	22	3	102, 407	1	2
TZMI102	0	6,003	−227			2	2
TZMI407	6,003	0	227			2	1
Mean	6,747	7,190	0	0			
Standard error[b]	394	394	299	240			

[a]SCA effects with tester TZMI407 are equal in magnitude but opposite in sign
[b]Standard error for tester GCA is 66 kg/ha

primers varied from two to four, with an average of 2.9. A total of 235 AFLP and 95 SSR polymorphic bands were detected in inbred lines from Cameroon, and 246 AFLP and 100 SSR polymorphic bands were found in inbred lines from Nigeria. Nearly 80% of the AFLP and 90% of the SSR alleles occurred at a frequency of less than 0.75 in the lines from each country. Only two AFLP alleles were specific to the inbred lines from Cameroon, while four AFLP and four SSR alleles were specific to the lines from Nigeria. The specific alleles occurred at a frequency of 0.28 or less in each set of lines.

Table 4 shows the GD estimates between pairs of lines, calculated for each marker system. The SSR data gave slightly higher GD values than did AFLPs. A broad range of GD values was found for pairs of lines bred in each country and for those bred in the two countries, for AFLP and SSR markers (Table 4) The average GD values for pairs of lines within each country and those between the two countries were similar for each marker system. The smallest minimum GD value was recorded between pairs of lines from Cameroon. Average GD values for combinations of the 38 inbred lines with TZMI102 were 0.46±0.07 for SSRs and 0.58±0.03 for AFLPs, and those with TZMI407 were 0.44±0.08 for SSRs and 0.35±0.04 for AFLPs.

The Mantel matrix correspondence test showed that the correlation coefficient between distance matrices of the AFLPs and SSRs was significant but low (r=0.29). As shown in Fig. 1, the dendrogram constructed using a distance matrix of each marker system subdivided the

Table 4 Average (± standard deviation), minimum and maximum values of genetic distance estimates among 40 mid-altitude inbred lines calculated from AFLP and SSR data

Combinations	Number of pairs of lines	Mean	Minimum	Maximum
	SSR			
Cameroon × Cameroon	153	0.49±0.11	0.04	0.71
Cameroon × Nigeria	396	0.51±0.08	0.30	0.73
Nigeria × Nigeria	231	0.48±0.08	0.23	0.73
All combinations	780	0.50±0.09	0.04	0.73
	AFLP			
Cameroon × Cameroon	153	0.38±0.07	0.02	0.48
Cameroon × Nigeria	396	0.40±0.06	0.20	0.64
Nigeria × Nigeria	231	0.40±0.07	0.19	0.64
All combinations	780	0.40±0.07	0.02	0.64

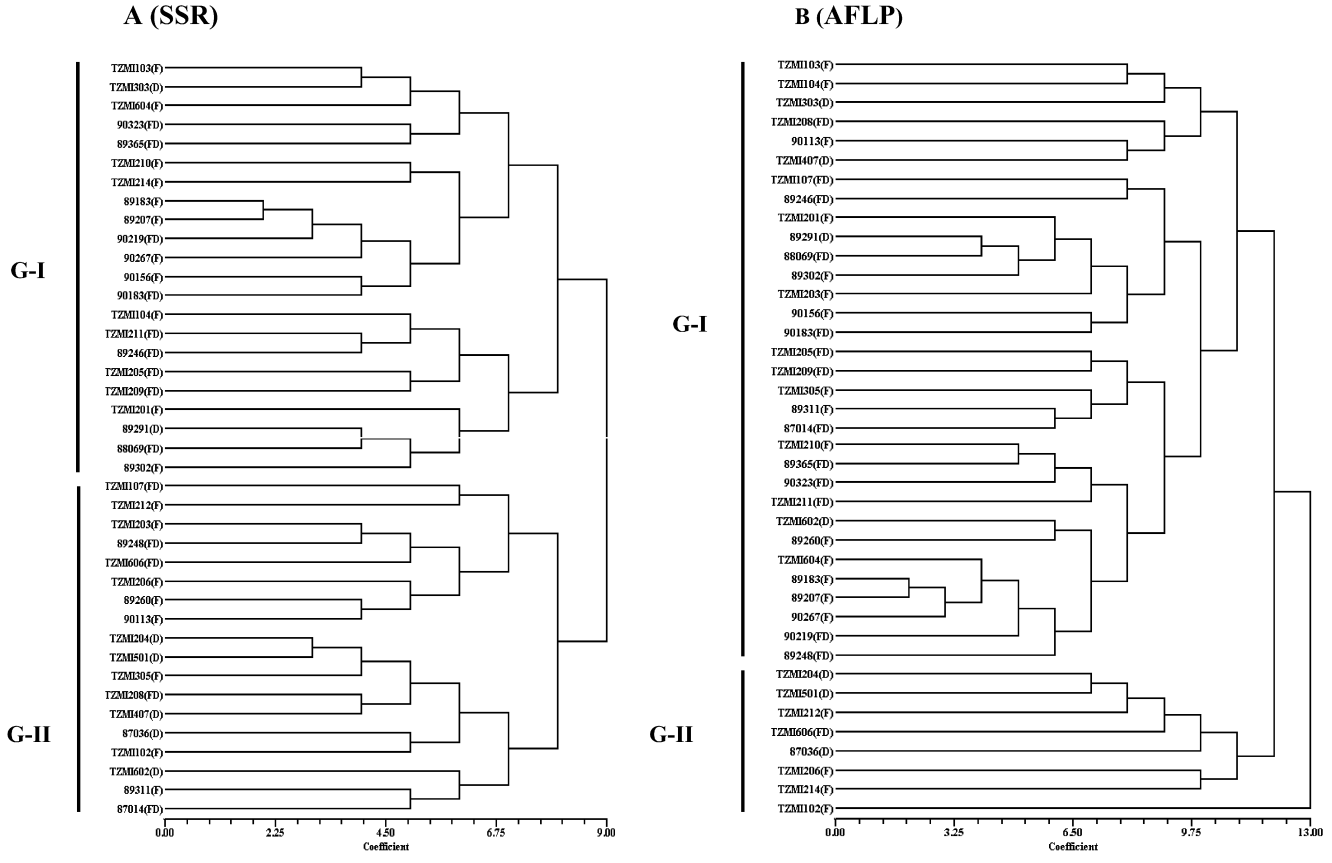

Fig. 1 Dendrogram of 40 mid-altitude inbred lines obtained using SSR (**A**) and AFLP (**B**) markers

lines into two major groups, with most of the lines included in the second group being from Nigeria. Although the number of lines clustered in each major group was different for the two markers, almost all the lines represented in the second AFLP group were also listed in the second SSR group. The inbred lines with different endosperm types did not form distinct groups based on the two marker systems.

Cluster analysis using the SSR GD matrix classified the 40 inbred lines into two main groups, with each cluster split further into two sub-groups (Table 1, Fig. 1). Viewing these associations from the top of the dendrogram, the first sub-group consists of lines most of which were bred in Cameroon. The second sub-group has a mixture of lines from the two breeding programmes. The third and fourth sub-groups consist of lines most of which were bred in Nigeria. The SSR markers placed the two testers in the same sub-group. Within each sub-group, inbred lines bred in the same country and/or with a common parentage cluster together (Table 1).

The dendrogram constructed with AFLP markers split the 40 maize inbred lines into two main groups, with the first group subdivided into two sub-groups (Table 1, Fig. 1). The first and third sub-groups consist of lines bred mainly in Nigeria, while the second group is a mixture of lines from the two countries, with most of them originating from the Cameroon breeding programme. The tester line, TZMI102, is an outlier from the sub-groups. Again,

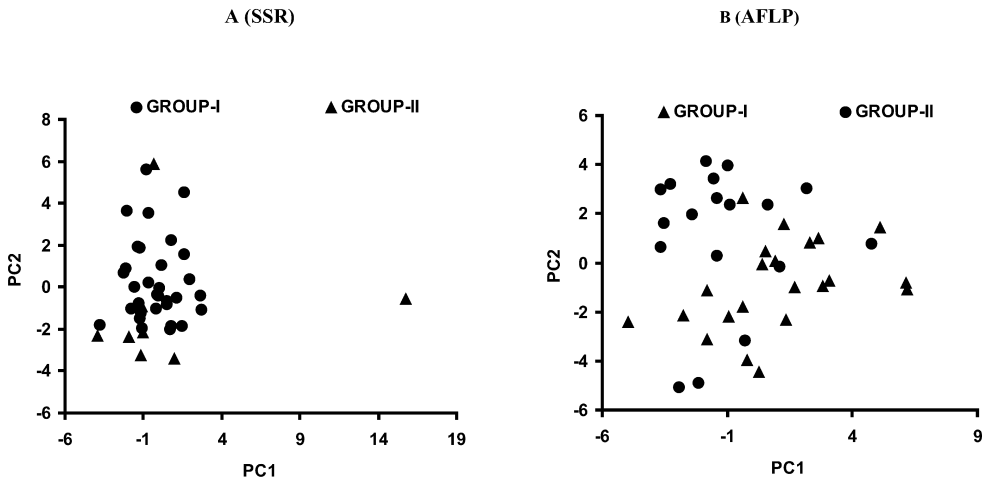

Fig. 2 Principal component analysis computed from a genetic distance matrix of 40 tropical mid-altitude inbred lines characterized using SSR (**A**) and AFLP (**B**) markers

inbred lines from the same breeding programme and/or with a common parentage tend to cluster together within each sub-group (Table 1).

The first and second principal component axes, termed PC1 and PC2, account for 22% and 13% of the total variation, respectively, in the AFLP data, while they explain 19% and 16% of the total variation, respectively, in the SSR data. As shown in Fig. 2, the two axes separate most of the mid-altitude maize inbred lines into two groups, which is consistent with the results obtained from the cluster analysis.

Correlation of marker-based genetic distance (GD) with SCA effect for grain yield

The correlation coefficients between GD estimates of the 38 inbred lines in combination with testers and their corresponding SCA effects for grain yield were not significant for each marker system (r=-0.10 to r=0.30). As shown in Table 3, the inbred lines assigned to the two yield-based heterotic groups (TZMI102 and TZMI407) were distributed widely in the two major groups of the two marker systems. The correlation among GD values of the 38 lines combined with TZMI102 was significant for SSRs (r=0.50, P=0.001) but not for AFLPs (r=0.31, P=0.06), while that with TZMI407 was significant for both SSRs (r=0.46, P=0.004) and AFLPs (r=0.49, P=0.001). Although all combinations of the 38 lines with TZMI102 had higher GD estimates than the corresponding combinations with TZMI407 for AFLPs (data not shown), the largest number of testcrosses having mean grain yields exceeding 7,000 kg/ha involved TZMI407 as a tester (Table 3).

Discussion

The relative performance of inbred lines in crosses with divergent testers of known origin has been commonly used to assign maize inbred lines into heterotic groups (Hallauer et al. 1988). The two inbred testers used in this study, TZMI102 and TZMI407, represent the flint and dent heterotic pattern, respectively, with good agronomic features and good combining ability. Even though the testers exhibited contrasting GCA effects, both of them, in more than 50% of the crosses made with the inbred lines, produced significantly higher yields than the hybrid between the two testers. These results suggest that the inbred lines evaluated in this study interacted positively for grain yield with the genetic backgrounds of the two testers and thus represent useful sources of favourable alleles for yield enhancement in the mid-altitude environments. These results also indicate that the testers have the capacity to uncover desirable alleles for grain yield and can therefore be used as potential testers in a maize hybrid-breeding programme for mid-altitude environments.

Significant GCA and SCA effects for grain yield were detected among the tropical mid-altitude inbred lines. The line × environment and tester × environment interactions were significant, possibly due to the differential reaction of the lines and testers to different disease pressure modulated by changes in climatic factors across years and locations. However, the SCA effect for grain yield, which was considered to be a major criterion for classifying the inbred lines, was consistent across environments. The testers were able to classify 23 of the 38 tested inbred lines into two heterotic groups based on SCA effects and testcross mean grain yields. This grouping was not related to the endosperm type of the inbred lines. As indicated by Messmer et al. (1992), it is no longer possible to classify lines as flint or dent based on endosperm type alone because new generations of lines with mixed origin are becoming available and breeders are attempting to eliminate the weaknesses of the flint germplasm by introducing dent germplasm. The outstanding performance of testcrosses of the remaining 15 inbred lines with the two testers indicates the presence of significant genetic diversity that may allow the assignment of the lines into more than two heterotic groups. As suggested by Kim et al. (1999), the diverse genetic backgrounds of the mid-

altitude inbred lines and their origin from source populations with mixed genetic composition may represent several potential heterotic groups that can be exploited for the development of high-yielding hybrids and synthetic varieties.

We found considerable genetic diversity among the inbred lines bred in Cameroon and Nigeria using both AFLP and SSR markers. Although most of the inbred lines from the two breeding programmes share common germplasm in their parentage (Kim et al., 1985; Everett et al. 1994a, 1994b), the average GD between lines from the two breeding programmes was as high as or higher than the average GD among lines within each breeding programme, possibly due to divergent selection for adaptation and other desirable agronomic features in each country. It is also noteworthy that inbred lines originating from the same breeding programme clustered together even when they share a common parentage with lines from the other breeding programme. Several generations of selection for similar desirable agronomic traits and resistance to diseases specific to each country may have contributed to more genetic uniformity of the inbred lines and thus to their clustering closer together. This strongly indicates that maize breeding programmes isolated in space can play a significant role in generating divergent inbred lines. This finding has a significant implication for maximizing heterosis in hybrids and synthetic varieties.

The molecular markers separated the diverse mid-altitude inbred lines that had no established heterotic pattern into groups of genetically similar lines. Unlike the yield-based analysis, which had classified 23 of the 38 tested inbred lines into two heterotic groups, the molecular marker-based analysis separated the 38 lines and the two testers into two groups, which were further subdivided into sub-groups consistent with the breeding history, origin and parentage of the lines. However, lines assigned to distinct heterotic groups on the basis of yield data did not form separate groups with the two marker systems. Contrary to our findings, diversity studies using AFLP and SSR markers in temperate maize germplasm have assigned inbred lines into heterotic groups established on the basis of empirical data (Ajmone-Marsan et al. 1998; Pejic et al. 1998; Senior et al. 1998; Lűbberstedt et al. 2000; Lu and Bernardo 2001; Enoki et al. 2002). On the other hand, Warburton et al. (2002) did not find good agreement between heterotic groups determined on the basis of testcross data and those generated using SSR markers for CIMMYT maize inbred lines, possibly due to the large genetic diversity present in the established heterotic groups.

The groups formed from marker data for the tropical mid-altitude inbred lines may not strictly represent very closely related lines. The high grain yields of several testcrosses within marker-based groups show that significant genetic diversity is still present within groups. Caution should, therefore, be exercised in classifying diverse maize inbred lines of mixed origin to heterotic groups based only on marker data. The use of marker-based clusters as a basis for selecting inbred lines from different groups in order to make crosses may improve the chance to generate productive hybrids. Lanza et al. (1997) and Parentoni et al. (2001) reported that the correlation between mean grain yields of single-cross hybrids and the marker-based GD estimates, which was low, became stronger when correlation analysis was performed based on the combination of lines belonging to different groups established by markers. The superior yield and agronomic performance of inter-group over intra-group crosses is also well documented in maize (Hallauer et al. 1988; Melchinger and Gumber 1998; Bernardo 2001). It is therefore suggested that marker-based GD estimates could form the basis for designing diallele or factorial crosses for field testing in order to establish consistent heterotic groups among the diverse tropical mid-altitude inbred lines. Clearly defined heterotic groups of the tropical mid-altitude maize inbred lines may develop following further field tests of crosses of lines derived from different marker-based groups.

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