DIVERSITY ANALYSIS OF ELITE MAIZE INBRED LINES ADAPTED TO WEST AND CENTRAL AFRICA USING SSR MARKERS

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ABSTRACT - Seventeen elite maize inbred lines of West and Central Africa adaptation with tropical and temperate x tropical origin were investigated for diversity at 18 SSR loci in non-coding regions of the maize genome, alongside two temperate inbred lines (B73 and Mo17), perennial teosinte (*Zea diploperennis*) and gamagrass (*Tripsacum dactyloides*). A total of 174 alleles were detected with a range of 5 to 15 alleles per maker and an average of 9.7 alleles per locus. Polymorphic information content (PIC) ranged from 0.29 in umc1226 to 0.92 in bnlg2122 with an average of 0.75. Relationships between heterotic groups and groups based on SSR data were quite varied for the lines studied. Primarily, the SSR markers grouped the lines on the basis of their origin, with three instances of a pair of heterotic lines clustering together; one pair of temperate origin and the other two tropical vs temperate x tropical. Four inbred lines (CMR 19, CMR 20, CMR 21, and CMR 26), belonging to three heterotic groups were, however, differentiated by SSR data. The markers showed potential for use in managing inbred lines germplasm adapted to West and Central Africa, particularly for classifying inbred lines for which records of ancestry are not readily available and for exploiting the heterosis known for tropical vs. temperate x tropical crosses.

KEY WORDS: Diversity; Heterotic groups; Maize inbred lines; West and Central Africa.

INTRODUCTION

Maize (*Zea mays* L.) is an important cereal crop in West and Central Africa (WCA). It is cultivated in all ecological zones of the sub-region. However, the ecological zones with the greatest potential for in-creased maize production are the moist savannas (1270-1590 mm annual rainfall), where there are relatively high solar radiation and low incidence of pests and diseases during the cropping season (BADU-APRAKU *et al.*, 2008). In the last two decades, the crop has witnessed a rising profile in the sub-region. This is evidenced by the inroad the crop has made into the drier Sudan savanna where sorghum and millet dominate, following the development of early maturing and drought-tolerant varieties. Consequently, the area cultivated to maize has been on the increase in WCA (FAO, 2007). Although open-pollinated varieties are more popular among farmers, there is a growing demand for hybrids to take advantage of heterosis. The Nigerian government provided seed money to the International Institute of Tropical Agriculture (IITA) for initiating inbred-hybrid development programme in 1979. Since then, there has been considerable effort directed to inbred-hybrid breeding in the sub-region.

As a result of their susceptibility to tropical pests and diseases, temperate germplasm were, in many instances, crossed with materials of tropical adaptation, and thereafter inbred lines were developed from the resulting populations. The inbred lines derived from these crosses were used to form hybrids tested extensively in several countries through regional trials in collaboration with National Agricultural Research Systems. While KIM and AJALA (1996) reported the combining ability of some tropical lowland germplasm in West Africa, MENKIR *et al*. (2003) provided information on the heterotic pattern of many of the lines investigated by KIM and AJALA (1996) as well as many other lines based on grain yield in well-watered and drought stress environments. Many of these lines are parents of commercial hybrids and sources of genes for resistance to biotic stresses in the sub-region (KIM *et al*., 1987).

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Knowledge about germplasm diversity and genetic relationships among breeding materials could be an invaluable aid in crop improvement strategies (MOHAMMADI and PRASANNA, 2003). DNA markers provide a direct measure of genetic diversity and go beyond diversity based on agronomic traits or geographic origin (DREISIGACKER et al., 2005), thus helping to better manage germplasm and develop more efficient strategies for crop improvement. Among DNA markers, simple sequence repeat (SSR) markers, a polymerase chain reaction (PCR)-based technique, have gained greater prominence because they are easy to generate, highly polymorphic and repeatable, easily detectable and relatively cheap (HECKENBERGER et al., 2002; MATSUOKA et al., 2002; KAPILA et al., 2008). No published information is available on the molecular diversity of the widely investigated maize lines in WCA reported by KIM and AJALA (1996) and MENKIR et al. (2003). This paper reports the diversity among some of these tropical inbred parents of hybrids cultivated in West and Central Africa and other lines developed in the subregion using SSR markers, and relates the SSR-based diversity to known heterotic groups.

MATERIALS AND METHODS

Seventeen inbred lines of diverse origin adapted to WCA were used for the study (Table 1). Of these, 10 inbred lines (TZi lines) were developed by the Maize Program of the International Institute of Tropical Agriculture (IITA) while seven (CMR lines) were from the Cameroon National Program. Two temperate inbred lines – B73 and Mo17, an ancestor and probable progenitor of maize – (perennial teosinte) Zea diploperennis, and a wild relative – gamagrass (Tripsacum dactyloides), were included in the study. The inbred lines were at advanced stages of inbreeding and many have been used as parents of commercial hybrids.

DNA was extracted from one plant for each of the genotypes, using a modified CTAB (cetyltrimethylammonium bromide) procedure (SAGHAI-MAROOF et al., 1984). Leaf samples were lyophilized for DNA extraction. Freeze-dried samples were ground at 800 strokes for 2 min with a 2000 Geno/Grinder (Spex CertiPrep Inc., Metuchen, NJ). A total of 18 unlinked dinucleotide repeat SSRs that had previously shown consistent stepwise mutation in assays of hundreds maize landraces and inbred lines (S.E. MITCHELL, unpublished) were selected for analysis. The loci are listed in Table 2. Primer sequences are available from the Maize Genetics Database (MaizeGDB) (http://www.agron.missouri.edu/ssr.html). Primer pairs for each locus, where one primer was labeled at the 5' end with FAM, VIC, PET or NED fluorescent dyes were obtained from Applied Biosystems (Foster City, CA). Multiplex PCRs were performed according to MATSUOKA et al. (2002) with minor modifications. Two to four SSR loci were assayed simultaneously in each reaction. The PCRs were performed in 20-μl volumes containing 30 ng of template DNA, 1 to 4 pmol of each of forward and reverse primers for each locus, 1X PCR buffer (Promega, Madison, WI), 0.2 mM dNTPs, 1.5 mM $MgCl_2$ and 1.0 U Taq polymerase (Promega). Reactions were performed with a PTC-225 Peltier Thermal Cycler (MJ Research Inc., Watertown, MA) using the amplification conditions of 95°C for 4 min; followed by 30 cycles of 95°C for 1 min, 55°C for 2 min and 72°C for 2 min; In the last cycle, extension time at 72°C was increased to one hour. Samples containing 0.5 μl of PCR products, 9 μl of formamide, 0.05 μl Genescan™ -5-LIZ™ size standard (Applied Biosystems) and 0.45 μl of distilled water were denatured at 95°C for 5 min and assayed on a capillary DNA sequencer (Applied Biosystems, model 3730 XL). Allele-calling was done using Genemapper 3. 0 (Applied Biosystems) and a custom Excel macro, kindly provided by Carlos Harjes, was used to bin the alleles. Allele sizes were exported as an Excel file and data analyzed using PowerMarker V3.25 (LIU, 2004). The software generated number of alleles and polymorphic information content (PIC) for each of the SSR loci. A distance matrix was generated from the data and subsequently grouped by UPGMA method using the NTSYS program version 2.02j (ROHLF, 1998). Principal Component Analysis (PCA) was performed with Genetix 4.05 (BELKHIR et al., 2004).

RESULTS

All the markers were polymporphic. The 18 SSR markers detected a total of 174 alleles ranging from 5 alleles per locus (umc1226) to 15 (bnlg2122) with an average of 9.7 alleles per locus (Table 2). Polymorphic information content (PIC) ranged from 0.29 in umc1226 to 0.92 in bnlg2122 with an average of 0.75. Percentage heterozygosity ranged from 5.6% in phi099 to 30.0% in bnlg1209 and umc1844. Five genotypes (CMR 19, CMR 23, TZi 9, TZi 18 and B73) were homozygous at the 18 SSR loci, while eight were heterozygous at one locus. The highest number of heterozygous loci was obtained in TZi 4 (12), followed by Tripsacum (11).

Genetic distance ranged from 0.47 to 1.00. A total of 14 pairs of lines (out of 210) had genetic distance of 1.0, indicating that they exhibited differences at the 18 SSR loci studied. The dendogram produced from the UPGMA showed four groups (Fig. 1). Mean within-group genetic distance was 0.71 while mean between-group genetic distance was 0.86. UPGMA clustering showed agreement with the PCA which separated Zea diploperennis and Tripsacum from the Zea mays lines on the first axis (figure not shown).

The first group (Group I) consisted of two temperate lines – B73 and Mo17, and TZi 11 – a line of temperate x tropical origin for which the temperate parent was Mo17. The second group (Group II) was mixed and had 10 lines made up of six TZi lines and four CMR lines. Six of the 10 lines were of tropical

TABLE 1 - *Parentage, origin and adaptation of inbred lines used in the study.*

Inbred line	Parentage	Origin	Adaptation
CMR 19	Pop 43 x TZBSR	Tropical	Lowland
CMR 20	NCRE 8401*	Tropical	Lowland
CMR 21	Pop 32 x TZMSR	Sub-tropical x Tropical	Mid-altitude
CMR 23	Suwan 1 x NCRE 8401*	Tropical	Lowland
CMR 24	Pop 32 x TZMSR	Sub-tropical x Tropical	Mid-altitude
CMR 25	Pop 43 x TZMSR	Tropical	Mid-altitude
CMR 26	Pop 43 x TZBSR	Tropical	Lowland
TZi 3	Across 7721 x TZSR	Tropical/African	Lowland
TZi 4	Guana Caste 7729 x TZSR	Tropical/African	Lowland
TZi 8	TZB x TXSR	Tropical/African	Lowland
TZi 9	Sids 7734 x TZSR	Tropical/African	Lowland
TZi 10	Tlalt. 7844 x TZSR	Tropical/African	Lowland
TZi 11	Mo17 x TZSR	Temperate x Tropical	Lowland
TZi 12	N28 x TZSR	Temperate x Tropical	Lowland
TZi 15	N28 x TZSR	Temperate x Tropical	Lowland
TZi 18	Sete Lag. 7728 x TZSR	Tropical/African	Lowland
TZi 25	B73 x TZRppSR	Temperate x Tropical	Lowland
B73	BSSS C5 (Iowa Stiff Stalk)	Temperate	
Mo17	CL 187-2 x C103	Temperate	
Tripsacum	Tropical		
Zea diploperennis	Tropical		

*NCRE 8401 = Pop 43 x BuLSR x TZPB x TZB.

TABLE 2 - *SSR marker information, number of alleles, polymorphic information content (PIC) and heterozygosity at SSR loci.*

SSR marker[a]	Bin	Repeat motif	No. of alleles	PIC	% Germpl. hetero.	Germpl. hetero.
umc1106	1.00	(GA)10	7	0.59	16.7	*Trips.*, TZi 10, *Zea diplo*
umc1774	1.10	(GT)7	7	0.76	15.0	CMR 25, TZi 4, Mo17
umc1934	2.02	(AT)8	12	0.85	16.7	CMR 25, *Trips.*, TZi 4
bnlg1018	2.04	(AG)16	9	0.89	10.5	CMR 25, TZi 4, TZi 8
umc1394	3.01	(AT)10	9	0.74	10.5	*Trips.*, *Zea diplo*
phi099	3.04	(AC)	6	0.76	5.6	CMR 26
umc1844	3.08	(TC)8	8	0.79	30.0	CMR 25, CMR 26, TZi 4, TZi 12
bnlg1182	3.09	(AG)19	9	0.75	19.0	*Trips.*, *Zea diplo*
umc1226	5.02	(GT)8	5	0.29	11.1	*Trips.*, TZi 4
bnlg1043	6.00	(AG)20	11	0.84	11.8	CMR 25, TZi 4
umc1883	6.00	(CT)8	9	0.67	23.4	CMR 20, CMR 26, *Trips.*, TZi 3,TZi 15
bnlg2271	7.03	(AG)15	7	0.58	10.0	*Trips.*, TZi 10
bnlg1131	8.09	(AG)17	9	0.83	16.7	CMR 25, TZi 4, *Zea diplo*
bnlg2122	9.01	(AG)17	15	0.92	20.0	CMR 26, *Trips.*, TZi 4, *Zea diplo*
umc1040	9.01	(CT)11	11	0.84	15.0	CMR 20, *Trips.*, TZi 4
bnlg1209	9.04	(AG)12	13	0.84	30.0	CMR 21, CMR 24, CMR 25, TZi 4 TZi 10, TZi 11
umc1866	10.03	(AT)7	11	0.81	21.1	CMR 21, CMR 25, TZi 4, *Trips.*
bnlg1074	10.05	(AG)14	13	0.78	15.0	TZi 4, *Trips.*, *Zea diplo*

[a] SSR information was obtained from the MaizeGDB; Germpl. Hetero. = germplasm heterozygous; *Trips = Tripsacum*; *Zea diplo = Zea diploperennis.*
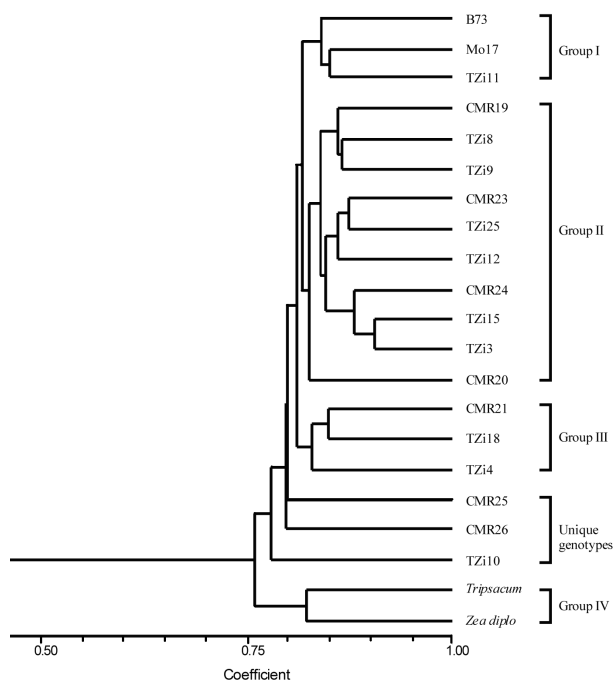
FIGURE 1 - UPGMA dendogram of 17 elite maize lines adapted to West and Central Africa with four checks (*Zea diploperennis*, *Tripsacum*, B73 and Mo17).

origin; three were of temperate x tropical origin while one was of subtropical x tropical origin. Five of the 10 lines either had TZSR as the parent or as one of the parents. The third group (Group III) consisted of two tropical lines, also derived from TZSR, and one other line with tropical x subtropical origin, while the fourth (Group IV) had *Zea diploperennis* and *Tripsacum*. Each of the three remaining inbred lines viz. CMR 25, CMR 26 and TZi 10 were considerably different from one another and the remaining *Zea mays* lines and did not form any cluster.

Relationships between known heterotic groups and groups based on SSR data were quite varied for the lines studied. B73 and Mo17, two temperate lines belonging to two heterotic groups clustered together in Group I. TZi 3 and TZi 15, two inbred lines belonging to two heterotic groups identified for WCA (Table 3) clustered in the same sub-group in Group II. Also, TZi 8 and TZi 9, both tropical in origin, that simultaneously belonged to the two heterotic groups recognized for TZi lines clustered in Group II. TZi 3 and TZi 10 belong to the same heterotic group but showed considerable diversity with the consequence that the two lines did not cluster together.

CMR 19 and CMR 20 belong to the same heterot-

ic group (Table 4) and also clustered in Group II. On the other hand, CMR 21, CMR 24 and CMR 25, all mid-altitude lines, which belong to the same heterotic group were differentiated with respect to the groups formed based on SSR data. CMR 19, CMR 21 and CMR 26 which belonged to different heterotic groups either belonged to different groups based on SSR data or failed to cluster. This is also true for CMR 20, CMR 21 and CMR 26.

DISCUSSION

The present study provides information on the molecular diversity of elite lowland and mid-altitude lines adapted to WCA. The average number of alleles per locus of 9.7 was higher than the values reported in other studies (PEJIC *et al.*, 1998; LU and BERNARDO, 2001; WARBURTON *et al.*, 2002) that used SSR makers to determine the genetic diversity of maize inbreds. One possible reason for this is the exclusive use of dinucleotide repeat SSRs in the present study. Di-repeat SSRs are known to yield a significantly higher number of alleles per marker than SSRs with longer repeat motifs (HECKENBERGER *et al.*, 2002). In addition, the SSRs were chosen in non-coding regions of the genome which are expected to be least biased by selection. The PIC obtained in this study was higher than the 0.59 reported for 70 SSR markers in 94 inbred lines representative of the genetic diversity among lines derived

TABLE 3 - *Known heterotic groups of TZi lines used in the study[a].*

Group 1	Group 2
TZi 12	TZi 3
TZi 15	TZi 10

[a] Summarised from MENKIR *et al.* (2003)
TZi 8 and TZi 9 belong to the two heterotic groups.

TABLE 4 - *Known heterotic groups of CMR inbred lines used in the study.*

Group 1	Group 2	Group 3
CMR 19	CMR 21	CMR 26
CMR 20	CMR 24	
	CMR 25	

from the Corn Belt Dent and Southern Dent Maize races (SENIOR *et al.*, 1998). These results, together with the high mean genetic distance among the genotypes, indicate considerable diversity among the WCA lines studied.

It was interesting to observe that TZi 4, a tropical lowland inbred line of the Tuxpeno group which over the years has shown consistent high GCA for yield in many locations in West Africa (KIM and AJALA, 1996; YALLOU, 2005) was heterozygous at 12 of the 18 microsatellite loci. The detection of more than one allele at some of the SSR loci for this and 13 of the other inbred lines could not have been due to residual heterozygosity, given that the inbred lines used in this study are at advanced stages of inbreeding and are parents of commercial hybrids. Since duplication is well documented in maize (HELENTJARIS, 1995), a possible explanation for the observed heterozygosity is the amplification of similar sequences in two separate genomic regions. Our results are similar to those of SENIOR *et al.* (1998) who out of 94 US Corn Belt inbred lines obtained two bands for 34, 40 and 11 inbred lines at SSR marker loci phi011, phi055 and phi096, respectively.

The grouping of *Zea diploperennis* and *Tripsacum dactyloides* in a cluster distinct from the *Zea mays* clusters is consistent with the known status as progenitor and wild relative, respectively, of cultivated maize. Although, lowland x mid-altitude germplasm has been identified as a heterotic pattern for the sub-region (KIM, 1997; C. THE, 2008, unpublished), the mid-altitude lines included in this study did not group together, indicating that the SSRs did not cluster the genotypes based on adaptation. While inbred lines of different heterotic groups clustered differently for some of the lines, especially the CMR lines, this was not always the case. In the present study, the clustering together of B73 and Mo17, two temperate lines representing the Reid Yellow Dent and Lancaster Sure Crop heterotic pattern that has been most widely exploited in the USA, as well as the pair of TZi 3 and TZi 15 that are known heterotic lines in WCA, indicate that the grouping produced by the SSR markers did not always follow heterotic groups based on grain yield. These results are in agreement with those of SENIOR *et al.* (1998) who reported that SSR markers grouped Reid and Lancaster lines in the same cluster. MENKIR *et al.* (2004) also reported that SSR markers placed the two heterotic testers for grouping mid-altitude inbred lines adapted to WCA in the same sub-group while MENKIR *et al.* (2006) observed

that in spite of the great genetic similarity between KUSR and L4001, two inbred lines adapted to WCA, the single cross between the two lines is a productive commercial hybrid marketed in Nigeria. Similarly, 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.

While the results of the present study indicate that the SSR markers did not consistently produce groups that align with heterotic classification, especially giving the mixed origin of the lines used, they indicate the usefulness of SSR markers in determining the relative composition of tropical and temperate germplasm, particularly in cases where such records are not readily available. This is best demonstrated by the clustering of TZi 11 with Mo17 and B73. Mo17 is one of the two parents of TZi 11. These results are in agreement with those of MATSUOKA *et al.* (2002) who reported that SSR markers show a principal division between tropical and temperate lines. Although three other temperate x tropical lines clustered with tropical lines in Group II, it is expected that a temperate x tropical line can cluster with temperate or tropical lines depending on whether the genome is more temperate than tropical or vice versa. Whenever clusters are non-overlapping, an inbred line that is related to two other inbred lines from separate clusters will only be grouped with the one to which it is more closely related. In WCA where crosses between temperate and tropical germplasm have been identified as one of the most promising heterotic patterns, the deployment of SSR markers would be of direct benefit in the development of this group of hybrids. The three inbred lines that did not cluster with any other viz. TZi 10, CMR 25 and CMR 26 are heterotic with inbred lines clustered in Groups II and III. These results can guide the selection of other pairs of crosses for yield evaluation, thus helping to better manage WCA maize germplasm.

BENCHIMOL *et al.* (2000) had earlier demonstrated the possibility of inbred lines derived from the same heterotic groups producing high yielding hybrids. For mid-altitude lines of West and Central Africa adaptation, GD estimates of 38 inbred lines in combination with testers and their corresponding SCA effects for grain yield were not significant for both AFLP and SSR markers (MENKIR *et al.*, 2004). PARENTONI *et al.* (2001) reported that the correlation between mean grain yields of single cross hybrids and marker-based GD estimates, which was low, be-

came stronger when correlation analysis was performed based on the combination of lines belonging to different groups established by markers. Results of the present study and those of MENKIR *et al.* (2004, 2006) indicate that two inbred lines can be heterotic without being genetically distant from one another. Similarly, two genetically distant lines are not necessarily heterotic. Inbred lines that are heterotic and genetically distant, in addition to high yield are expected to be more stable. GRAUFFRET *et al.* (2000) reported that temperate x tropical highland hybrids were more stable across diverse growing environments than temperate x temperate or tropical x tropical hybrids, a result the authors attributed to broader adaptation to diverse production environments. Consequently, data on molecular diversity have potential for use in addressing this aspect of crop performance.

The *Zea mays* lines used in the present study resulted from two breeding programs in WCA. The opportunity for better management of maize germplasm offered by SSR markers can provide a platform for greater exchange and use of maize inbred lines for greater productivity in the sub-region.

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