Genetic diversity of African maize inbred lines revealed by SSR markers

B. W. LEGESSE[1,2], A. A. MYBURG[2], K. V. PIXLEY[3] and A. M. BOTHA[2]

[1]*Ethiopian Agricultural Research Organization (EARO), Addis Ababa, Ethiopia*
[2]*Department of Genetics & Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa*
[3]*Tropical Ecosystem Directorate offices for Maize Improvement. CIMMYT, Int. Col, El Batan, Texcoco, Edo. De Mexico, Mexico*

Legesse, B. W., Myburg, A. A., Pixley, K. V. and Botha, A. M. 2006. Genetic diversity of African maize inbred lines revealed by SSR markers. — *Hereditas 144*: 10–17. Lund, Sweden. eISSN 1601-5223. Received June 22, 2005. Accepted August 23, 2006

Knowledge of genetic diversity (GD) and relationships among maize inbred lines is indispensable in a breeding program. Our objectives were to (1) investigate the level of genetic diversity among maize inbred lines and (2) assess their genetic structures by applying simple sequence repeat (SSR) markers. Fifty-six highland and mid-altitude maize inbred lines obtained from CIMMYT programs in Ethiopia and Zimbabwe were genotyped using 27 SSR loci. All of the genotypes studied could unequivocally be distinguished with the combination of the SSRs used. In total, 104 SSR alleles were identified, with a mean of 3.85 alleles per locus. The average polymorphism information content (PIC) was 0.58. GD expressed as Euclidean distance, varied from 0.28 to 0.73 with an average of 0.59. Cluster analysis using unweighted pair group method with arithmetic average (UPGMA) suggested five groups among the inbred lines. Most of the inbred lines adapted to the highlands and the mid-altitudes were positioned in different clusters with a few discrepancies. The pattern of groupings of the inbred lines was mostly consistent with available pedigree information. The variability detected using SSR markers could potentially contribute towards effective utilization of the inbred lines for the exploitation of heterosis and formation of genetically diverse source populations in Ethiopian maize improvement programs.

A. M. Botha, Department of Genetics & Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, 0002 Pretoria, South Africa. E-mail: ambotha@postino.up.ac.za

Maize is one of the well-known cereals in its adaptability and importance worldwide. In Ethiopia, it is adapted in different agro-ecological zones. Approximately more than 80% of the country's annual maize production is obtained from the mid-altitude and the highland zones. A significant portion of the small-scale farmers living in these zones depend on maize production for different purposes. It is used as human food, animal feed, and means of income and source of employment for an overwhelming majority of the population (EARO 2000). Despite this, most of the varieties currently grown in the highlands are low yielding local cultivars. The maize breeding program in Ethiopia lacks genetically diverse source material and has had little success developing high yielding hybrids and improved open-pollinated varieties for highlands.

Knowledge on the genetic diversity and relationships among maize inbred lines is indispensable to identify promising combinations for exploitation of heterosis and establishment of heterotic groups for use as source materials in a breeding program. Molecular markers have proven valuble for genetic diversity analysis of many crop species. Their expression, unlike morphological markers, is not influenced by environmental factors; hence reflect the actual level of genetic difference existing between genotypes (SMITH and SMITH 1992; WESTMAN and KRESOVICH 1997). Microsatellites or simple sequence repeats (SSRs) are DNA markers with short stretches of tandemly repeated di-, tri- or tetra-nucleotide motifs (WEBER 1990). SSRs are characterized by a great abundance (MATSUOKA et al. 2002), high variability (TAUTZ 1989; SCHUG et al. 1998) and even distribution throughout a wide range of genomic regions (LIU et al. 1996; SENIOR et al. 1996). They are co-dominant, highly polymorphic, multi-allelic and have become the marker of choice for genetic analysis in crops (GUPTA and VARSHNEY 2000). In maize, microsatellites have proved to be a valuable tool for genome mapping (TARAMINO and TINGEY 1996), population and conservation genetics studies (POWELL et al. 1995), property right protection (KUBIK et al. 2001), marker-assisted selection (WEISING et al. 1998) and diversity measurements (WARBURTON et al. 2002; PINTO et al. 2003). They have also proved useful for designation of lines into heterotic groups (ENOKI et al. 2002). SSRs can provide greater power of discrimination than restriction fragment length polymorphism (RFLP) markers and can reveal genetic associations

DOI: 10.1111/j.2006.0018-0661.01921.x

that are reflective of the pedigree of the inbred lines (Smith et al. 1997; Pejic et al. 1998; Yuan et al. 2000). SSR technology is dependent on polymerase chain reaction (PCR); therefore, polymorphism can be detected by using the less costly and more widely available agarose gel system (Senior et al. 1998; Bantte and Prasanna 2003; Pinto et al. 2003).

In this study, maize inbred lines obtained from CIMMYT research programs in Ethiopia and Zimbabwe were genotyped using SSR markers. DNA polymorphisms were detected by separation in agarose gel. The objectives of the study were to (1) investigate the level of genetic diversity among maize inbred lines and, (2) assess their genetic structures based on cluster analysis with the aim to generate broad-based source germplasm for the highland maize breeding program in Ethiopia.

MATERIAL AND METHODS

Plant material

Fifty-six maize inbred lines, comprised of 35 lines from the highland maize breeding program in Ethiopia and 21 mid-altitude inbred lines from CIMMYT-Zimbabwe were studied. Of the 35 Ethiopian-adapted lines, 32 were from CIMMYT (Ethiopia) and were developed from three populations: 1) Kitale Synthetic II × N3-type lines, 2) Ecuador-573 × SC-type lines, and 3) Pool9A × IITA's mid-altitude streak resistant population. They were introduced to Ethiopia in 1997, and were selected for vigour, disease resistance and adaptaptablity for the highland. The remaining three inbred lines are successful line testers in the mid-altitude and highland transition maize-breeding program of Ethiopia. The inbred lines from CIMMYT-Zimbabwe are tolerant or resistant against maize streak virus, gray leaf spot (*Cercospora zeae-maydis* Tehon) and northern leaf blight (*Exserohilum turcicum*) (Pixley and Zambezi 1996; Schechert et al. 1999; Welz et al. 1999). These diseases are important in various maize regions including highlands in Ethiopia. The pedigree of these lines is given in Table 1.

DNA isolation

Leaf tissue for each inbred line was harvested from three-to four- week old seedlings. Genomic DNA was isolated using hexa-decyltrimethyl-ammonium bromide (CTAB) DNA extraction procedure, as described by Doyle and Doyle (1987). Briefly, about 50–70 mg of leaf tissue from each inbred line was sampled into a 2 ml screw-cap tube containing 800 µl of 5% CTAB extraction buffer. The leaf tissues were homogenized into fine pastes using a FastPrep^{R} FP120 Instrument (QBiogene, Carlsbad, CA, USA) and then incubated at 65°C for 60 min. The samples were extracted once with chloroform iso-amyl alcohol (24:1) and precipitated twice with 70% and 95% ethanol. The pellets were air dried and re-suspended in 100 µl TE (10 mM Tris-HCl, pH 8.0 and 0.2 mM EDTA). DNA concentration was estimated by comparison to a serial dilution of lambda DNA standard in a 1% agarose gel and adjusted to approximately 10 ng per µl.

Primers and PCR amplification

The SSR primers used in the current study were obtained and chosen based on repeat units and bin locations to provide uniform coverage of the entire maize genome. A total of 27 informative SSR loci were chosen after initial screening of 105 SSR markers using a sample of eight inbreds. The selected SSRs included at least one SSR locus from each maize chromosome. PCR reactions were carried out following the Missouri Maize SSR protocol in a 20 µl volume containing 50 ng genomic DNA, 1 × PCR buffer (10 mM Tris-HCl, pH 8.3), 0.4 mM of each dNTP, 1.0 mM $MgCl_2$, 0.4 µM forward and reverse primers and 0.5 U Ampli-Taq DNA polymerase (Promega). The amplification consisted of a denaturing step of 2 min at 94°C with subsequent touchdown profile. The first part of the profile was performed for 10 cycles (0.30 min at 94°C; 0.45 min at 65 –1°C per cycle) and followed by 30 cycles (0.30 min at 94°C; 0.30 min at 55°C; 1.30 min at 72°C) of denaturing, annealing and extension steps, respectively. A final step of one cycle (15 min at 72°C) was performed. Reactions were stopped with 2 µl loading-dye (500 µl ml^{-1} glycerol, 20 mM EDTA, 0.6 mg ml^{-1} bromophenol blue). Amplification products were separated by electrophoresis in a horizontal gel system (Bio-Rad, model 96) at 110V for 2.0 h on 3% agarose gels (50% agarose METAPHOR FMC Bio products: 50% agarose Gibco BRL) stained with ethidium bromide (0.5 µg ml^{-1}) using 0.5 × TBE buffer (89 mM Tris, 89 mM borate, 2 mM EDTA pH 8.3). Finally, the gels were photographed under UV light (Geldoc Bio-Rad) attached to a gel documentation system with Biocapture software (Vilber Lourmat, France).

SSR data analysis

Gel photographs were scored manually. The bands were binary coded by 1 or 0 for their presence or absence in each genotype. The assay efficiency index referred to as polymorphism information content (PIC) was calculated following the formulae applied by Smith et al. (1997). Estimates of similarity among all pairs of the

Table 1. *Identification and pedigree records of maize inbred lines assayed for genetic diversity using SSR loci.*

No.	Identification	Pedigree	Origin
1	Amboon6-1	KIT/SNSYN ((N3) TUXC1F1 # # (GLS = 1) 6-1	CIMMYT
2	Amboon6-3	KIT/SNSYN ((N3) TUXC1F1 # # (GLS = 1) 6-3	CIMMYT
3	Amboon6-4	KIT/SNSYN ((N3) TUXC1F1 # # (GLS = 1) 14-1	CIMMYT
4	Amboon6-6	KIT/SNSYN ((N3) TUXC1F1 # # (GLS = 1.5) 7-3	CIMMYT
5	Amboon6-8	KIT/SNSYN ((N3) TUXC1F1 # # (GLS = 1) 7-3	CIMMYT
6	Amboon6-9	KIT/SNSYN ((N3) TUXC1F1 # # (GLS = 1) 11-1	CIMMYT
7	Amboon6-10	KIT/SNSYN ((N3) TUXC1F1 # # (GLS = 1) 33-2	CIMMYT
8	Amboon6-14	KT/SNSYN ((N3) TUXC1F1 # # (GLS = 1) 11-2	CIMMYT
9	Amboon6-15	KIT/SNSYN ((N3) TUXC1F1 # # (GLS = 1) 14-2	CIMMYT
10	Amboon6-20	SRSYN95 ((KIT/N3) TUXF1 # # (GLS = 1) 6-1	CIMMYT
11	Amboon6-21	ECU/SNSYN (SC/ETO) C1 F1 ## (GLS = 1.5) 16-1	CIMMYT
12	Amboon6-22	ECU/SNSYN (SC/ETO) C1 F1 ## (GLS = 2.0)-3-1	CIMMYT
13	Amboon6-23	ECU/SNSYN (SC/ETO) C1 F1 ## (GLS = 2.0)-8-2	CIMMYT
14	Amboon6-25	ECU/SNSYN (SC/ETO) C1 F1 ## (GLS = 2.5)-24-2	CIMMYT
15	Amboon6-26	ECU/SNSYN (SC/ETO) C1 F1 ## (GLS = 2.5)-42-3	CIMMYT
16	Amboon6-27	ECU/SNSYN (SC/ETO) C1 F1 ## (GLS = 3.0)-23-1	CIMMYT
17	Amboon6-29	ECU/SNSYN (SC/ETO) C1 F1 ## (GLS = 3.5)-41-1	CIMMYT
18	Amnoon6-34	SRSYN95 ((ECU/SC/ETO) F1# # (GLS = 2)-18-2	CIMMYT
19	Amboon6-37	SRSYN95 ((ECU/SC/ETO) F1# # (GLS = 3)-21-1	CIMMYT
20	Amboon6-38	SRSYN95 ((ECU/SC/ETO) F1# # (GLS = 3.5)-40-1	CIMMYT
21	Amboon6-39	SRSYN95 ((ECU/SC/ETO) F1# # (GLS = 3.5)-4-2	CIMMYT
22	Amboon6-40	SRSYN95 ((ECU/SC/ETO) F1# # (GLS = 3.5)-39.1	CIMMYT
23	Amboon6-41	POOL9AC-7-SR (BC2) FS-1-1-3-1	CIMMYT
24	Amboon6-42	POOL9AC-7-SR (BC2) FS-1-4-2-3	CIMMYT
25	Amboon6-44	POOL9AC-7-SR (BC2) FS-4-3-SR-1-1	CIMMYT
26	Amboon6-47	POOL9AC-7-SR (BC2) FS-50-1-2-3	CIMMYT
27	Amboon6-49	POOL9AC-7-SR (BC2) FS-89-2SR-1-1	CIMMYT
28	Amboon6-51	POOL9AC-7-SR (BC2) FS-123-2-1-3	CIMMYT
29	Amboon6-54	POOL9AC-7-SR (BC2) FS-170-4-1-3	CIMMYT
30	Amboon6-58	POOL9AC-7-SR (BC2) FS-222-4-1-3	CIMMYT
31	Amboo6-59	POOL9AC-7-SR (BC2) FS-232-4-1-3	CIMMYT
32	Amboon6-60	POOL9AC-7-SR (BC2) FS-48-1-1-3	CIMMYT
33	142 –1-e	Unknown (derived from Ecuador –573)	Ethiopia
34	F7215	Unknown (derived from Kitale Syn. II)	Ethiopia
35	POOL 9A-MHM	Unknown (derived from Pool9A)	Ethiopia
36	CML202	ZSR923-S4BULK-5-1-BBB	CIMMYT
37	CML204	[7794]-SELF-4-1-S9-1-4-7-4-5-BBB	CIMMYT
38	CML206	[EV7992#/EVPOP44-SRBC3]#BF37SR-2-3SR-2-4-3-BBB	CIMMYT
39	CML216	[MSR:131]-3-3-3-5-BBB	CIMMYT
40	CML312	[S89500F2-2-2-1-1-B*5	CIMMYT
41	CML386	[EV7992#/EVPOP43-SRBC3]#b#bsr-118-2-2-5-7-B-1-1-B*4	CIMMYT
42	CML387	[EV7992#/EV8449-SR] C1F2-334-1(OSU8i)-1-1-X-X-3-BB	CIMMYT
43	CML388	[EV7992#/EV8449-SR] C1F2-334-1(OSU9i)-8-2(I)-X-1-2-BB	CIMMYT
44	CML389	[EV7992#/EV8449-SR]C1F2-334-1(OSU9i)-8-6(I)-X-X-3-BB	CIMMYT
45	CML390	[EV7992] C1F2-430-3-3-3-X-7-BB	CIMMYT
46	CML391	[EV7992] C1F2-430-3-3-X-1-BB	CIMMYT
47	CML392	[M37W/100MSR//SR52/ZAMXSR7794-4-3]#B-111-1-5-B*5	CIMMYT
48	CML393	[R201/TZMSRW]#B-18-1-1-3-2-X-1-BB	CIMMYT
49	CML394	[PL31/POOL16SR//PL9A] C1F2-124-2-X-X-X-BB	CIMMYT
50	CML395	90323(B)-1-X-1-B-B-1-1-B-B-1-1-B	CIMMYT
51	CML440	G16SeqC1F47-2-1-2-1-BBBBB	CIMMYT
52	CML441	ZM605C2F1-17-1-B-1-BB	CIMMYT
53	CML442	M37W/ZM607#bF37sr-2-3sr-6-2-X]-8-2-X-1-BBBB	CIMMYT
54	CML443	[AC8342/IKENNE {1} 8149SR//PL9A] C1F1-500-4-X-1-1-BB-1BB	CIMMYT
55	CML444	P43C9-1-1-1-1-1-BBBBB	CIMMYT
56	CML445	[[TUXPSEQ] C1F2/P49-SR]F2-45-7-5-1-B	CIMMYT

lines were calculated from the data matrices in the form of dissimilarity units and expressed as Euclidean genetic distance (Hintze 1998). Cluster analysis was performed to generate a dendrogram using the unweighted pair group method with arithmetic average (UPGMA) as implemented in the NCSS software (Hintze 1998).

RESULTS

Twenty-seven SSR primer sets identified 104 alleles among 56 maize inbred lines, and the numbers of allele scored for SSR loci ranged from two to seven, with mean of 3.85 alleles. A number of loci (umc2038, Phi115, umc1296, phi032) revealed two alleles, and the maximum number of alleles was detected at the Bnlg 182 locus. Representatives of microsatellite bands are shown in Fig. 1. The PIC values of the SSR loci ranged from 0.31 to 0. 71 with mean of 0.55 (Table 2). Ten SSR loci (umc1568, bnlg182, phi 037, bnlg108, nc003, umc2214, bnlg238, bnlg619, phi054, bnlg2190) manifested PIC values more than 0.6 indicating their potential informativeness to detect differences among the inbred lines. The pairwise genetic dissimilarity values determined using Euclidean distance ranged from 0.28 (CML 388 × CML389) to 0.73 (Ambo6-34 × CML204) with overall mean of 0.59. Cluster analysis showed a good fit to the data matrix as indicated based on relatively high cophenetic correlation coefficient value (0.76). Cophenetic correlation coefficient values greater than 0.75 lies within an acceptable range, hence ensures the reliability and consistency of the dendrogram with the distance matrices (Bohn et al. 1999).

The resulting dendrogram indicated that almost all of the inbred lines could be distinguished and clustered into five groups (Fig. 2). Cluster I, consisted of mid-altitude and highland inbreds. The highland inbred lines in this group share common ancestral origin, Pool9A, and constitute a larger portion of the cluster than the mid-altitude inbreds. In cluster II, the mid-altitude inbred lines from CIMMYT were grouped, with two sub-divisions in the main group. Cluster III, contained highland and mid-altitude inbreds. Most of the highland inbred lines in cluster III are related to Ecuador 573. Cluster IV is the largest group, all of which are highland adapted maize inbred lines. This group was further sub-divided into two subgroups mainly based on pedigree relatedness. Cluster V consisted of a mixed group of mid-altitude and highland inbreds.

DISCUSSION

Genetic diversity of the studied materials is the most important factor limiting average number of alleles identified per microsatellite locus during screening. However, other factors such as (1) number of SSR loci and repeat types and, (2) the methodologies employed for detection of polymorphic markers have been reported influencing allelic differences. In this work, the average number of alleles (3.85) and the number of SSR loci (27) used to screen the inbred lines were considerably lower than those reported previously in maize. Warburton et al. (2002) with 85 SSR loci found 4.9 alleles and Vaz Patto et al. (2004) determined 5.3 alleles using 80 SSR loci. However, our value (3.85) closely agreed with the findings reported by Bantte and Prasanna (2003) and Pinto et al. (2003), who reported 3.25 alleles using 36 SSR loci and 4.16 alleles with 30 polymorphic loci, respectively. These investigators also used the agarose gel system to screen the microsatellite loci in maize.

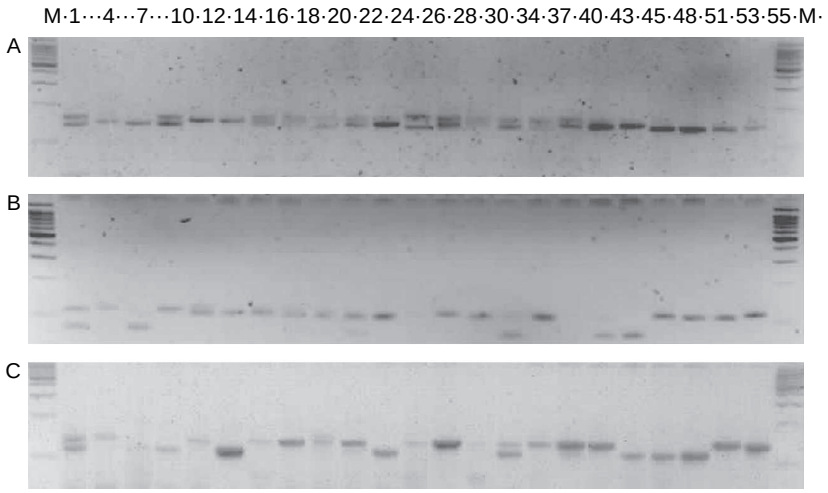

Fig. 1. Microsatellite markers of maize inbred lines. Amplifications performed with the primer sets umc1568 (**A**), bnlg108 (**B**) and bnlg602 (**C**) using genomic DNA from highland and mid altitude adapted inbred lines. A 100 bp ladder is shown in the left and right hand side of the gels. The numbers in the gel correspond to the identification of the inbred lines as indicated in Table 1 in the test.

Table 2. *Characteristics of each locus: repeat types, bin no., no. of alleles and polymorphic information content (PIC).*

SSR locus	Repeat types	Bin no.[a]	No. alleles	PIC value
Umc1568	TCG	1.02	3	0.69
Phi109275	AGCT	1.03	4	0.40
Bnlg 176	–	1.03	4	0.45
Bnlg182	–	1.03	7	0.68
Phi 037	AG	1.08	4	0.66
Bnlg108	–	2.04	4	0.64
Nc003	AG	2.06	6	0.70
Phi427434	ACC	2.08	3	0.43
Umc2214	CTT	2.1	5	0.63
Bnlg602	–	3.04	5	0.56
Umc2050	CGC	3.07	3	0.35
Umc1669	AGA	4.01	4	0.34
Ph1079	AGATG	4.05	3	0.35
Umc2038	GAC	4.06	2	0.50
Umc2036	CTC	5.01	3	0.44
Phi085	AACGC	5.06	3	0.60
Umc1153	TCA	5.09	4	0.56
Bnlg238	–	6	3	0.66
Umc1296	GGT	6.07	3	0.49
Phi034	CCT	7.02	5	0.57
Phi115	AT/ATAC	8.03	2	0.38
PhiI015	AAAC	8.08	3	0.58
Phi032	AAAG	9.04	2	0.31
Umc1367	CTG	9.05	3	0.60
Bnlg619	–	9.07	5	0.71
Phi054	AG	10.03	4	0.63
Umc1677	GGC	10.05	3	0.60
Bnlg2190	AG) 31	10.06	5	0.68
Mean			3.85	0.58

[a] =location of allele in the chromosomes of maize genome.

The average PIC value determined in our investigation agreed well with the earlier findings reported based on SSR marker in maize inbred lines (Senior et al. 1998; Heckenberger et al. 2002; Vaz Patto et al. 2004). PIC demonstrates the informativeness of the SSR loci and their potential to detect differences among the inbred lines based on their genetic relationships. Dinucleotide SSR loci (phi 037, nc003, bnlg619, phi054) identified the largest mean number of alleles (4.8) and mean PIC (0.67) as compared to tri, tetra and penta nucleotide repeats in this study, which is also in close agreement with previous observations for maize (Smith et al. 1997; Senior et al. 1998; Enoki et al. 2002).

In this work, agarose gel electrophoresis was used for the screening of the microsatellites since, compared to polyacrylamide gel or automated analysis; this is the most appropriate technology for routine analysis and also is a less costly and more widely available gel system. However, it is possible that an automated detection system would be able to resolve allelic variation better than gel electrophoresis analysis, and consequently, the number of alleles obtained would even be higher than that reported in this study. This may be particularly important for SSR loci containing dinucleotide repeats whose amplification products are in the 130 to 200 bp range, because PCR products differing by two base pairs cannot be resolved with agarose gel (Senior et al. 1998; Sibov et al. 2003).

Several inbred lines revealed more than one band during amplifications, which may have resulted from the co-dominant nature of the SSR markers (Bantte and Prasanna 2003). This was particularly evident in the highland maize inbred lines, which revealed high frequencies of double bands (Fig. 1). Similar results have been previously reported in maize inbred lines (Helentjaris et al. 1988; Senior et al. 1998; Matsuoka et al. 2002; Liu et al. 2003). These investigators speculated a number of probable causes for the occurrence of double bands in maize, including residual heterozygosity, pollen or seed contamination, mutation at specific SSR loci, or amplification of similar sequences in different genomic regions due to duplication. The most plausible explanation for obtaining such results in our case could be residual

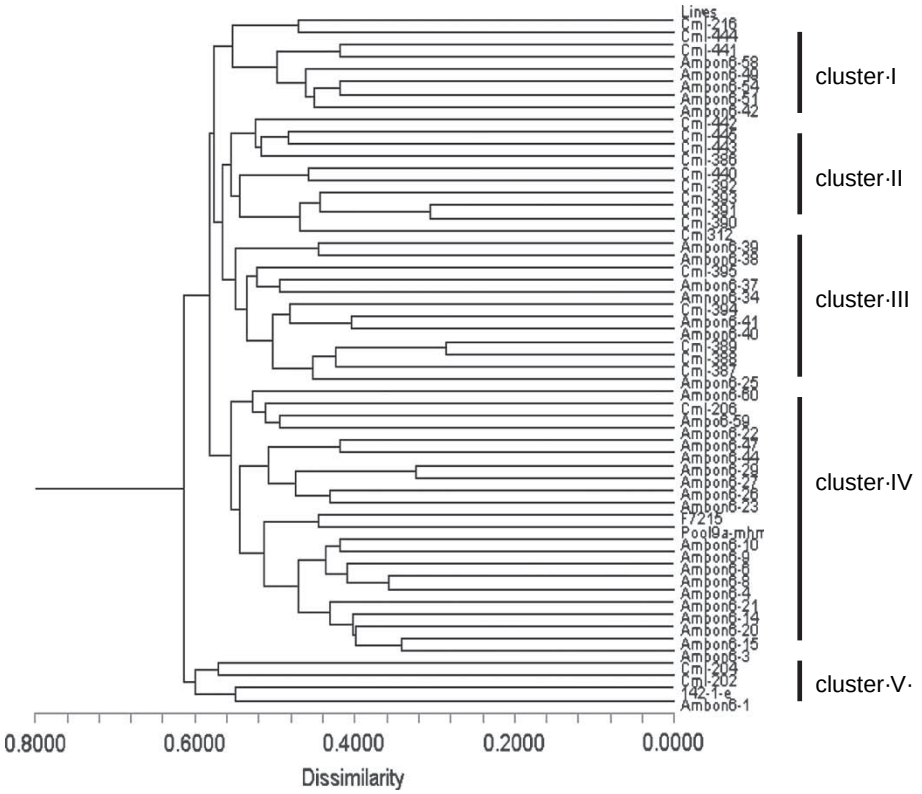

Fig. 2. Dendrogram derived using UPGMA cluster analysis based on SSR genetic distance data of 56 maize inbred lines. I, II, III, IV and V indicate major cluster groups.

heterozygosity, as a result of small number of generations of inbreeding (S_4) in the highland inbred lines.

The ability to provide distance measures between the inbred lines that reflect pedigree relatedness ensures a more stringent evaluation of the adequacy of a marker profile data. The fact that minimum genetic distance revealed between CML 388 and CML 389 (0.29) is a good indication confirming the power of SSR markers to distinguish between closely related inbred lines (Smith et al. 1997). The average genetic diversity existing among all the inbred lines was relatively high (59%), indicating that high levels of polymorphisms in the inbreds. These results are in close agreement with the findings reported in maize inbred lines using a SSR marker system (Smith et al. 1997; Senior et al. 1998; Vaz Patto et al. 2004).

The dendrogram constructed using the UPGMA clustering algorithm grouped the inbred lines into five clusters, which may be generalized into three main clusters representing the highland, the mid-altitude and the mixed group lines. These groupings, in most instances, revealed evidence of associations related to their pedigree records. This is in agreement with earlier investigators (Smith et al. 1997; Senior et al. 1998; Reif et al. 2003), who demonstrated the correspondence of SSR marker distance with pedigree information in maize. Alternatively, groupings of the inbred lines based on their adaptation regimes (cluster I and IV) were also evident based on the cluster analysis. This phenomenon could be in line with the findings of Jiang et al. (1999), who investigated the genetic bases for adaptation differences between highland and lowland tropical maize genotypes, and indicated the relative importance of genomic segments and their effects on adaptation to thermally diverse locations. Despite this, the mixed groups that included inbred lines from the highland and the mid-altitude adaptation regimes showed some tendency of fluctuation from available pedigrees. The inbred lines under investigation were previously developed at the same breeding center. Inadvertent genetic admixtures or incomplete pedigree records sometimes encounter in breeding programs. Such discrepancies are not uncommon when comparing molecular results with classification based on pedigree information. Effects of selection, drift and mutation on the DNA markers or human error might be the cause for these discrepancies (Warburton et al. 2002). Secondly, clustering processes that are not overlapping can be an alternative cause, because an inbred line that is related to two other inbred lines from

separate clusters will be grouped with one to which it is most closely related (AJMONE-MARSAN et al. 2001; SENIOR et al. 1998).

Overall, this study indicated that SSR markers largely separated the inbred lines into different clusters, which generally agreed with their pedigree records and adaptation regimes. Most of the inbred lines included in this study could be differentiated with the combination of primer pairs used, indicating the robustness of SSR markers for diversity analysis and heterotic groupings. High overall genetic variability was detected (0.59) among all inbred line combinations suggesting the opportunity to exploit the inbred lines for the development of varieties and formation of heterotic populations used to derive promising inbred lines.

Acknowledgements – This study is part of a PhD research project of the senior author conducted with financial support from the government of Ethiopia and the University of Pretoria. All kinds of support provided by the University of Pretoria, NRF of the South African government and CIMMYT are acknowledged

REFERENCES

Ajmone-Marsan, P., Gorni, C., Chitto, A. et al. 2001. Identification of QTL for grain yield and grain related trait of maize (*Zea mays* L.) using an AFLP map, different testers and cofactor analysis. – Theor. Appl. Genet. 102: 230–243.

Bantte, K. and Prasanna, B. M. 2003. Simple sequences repeat polymorphism in quality protein maize (QTL) lines. – Euphytica. 129: 337–344.

Bohn, M., Utz, H. F. and Melchinger, A. E. 1999. Genetic similarities among winter wheat cultivars determined on the bases of RFLPs, AFLPs SSRs and their use in predicting progeny variance. – Crop Sci. 39: 228–237.

Doyle, J. J. and Doyle, J. L. 1987. Genomic plant DNA preparation from fresh tissue-the CTAB method. – Pytochem. Bull. 19: 11.

EARO 2000. Research strategy for maize. Ethiopian Agric. Res. Org. (EARO), Addis Ababa, Ethiopia.

Enoki, H., Sato, H. and Koinuma, K. 2002. SSR analysis of genetic diversity among maize inbred lines adapted to cold regions of Japan. – Theor. Appl. Genet. 104: 1270–1278.

Gupta, P. K. and Varshney, R. K. 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. – Euphytica 113: 163–185.

Heckenberger, M., Bohn, M., Zeigle, J. S. et al. 2002. Variation of DNA fingerprints among accessions within maize inbred lines and implications for identification of essentially derived varieties I. Genetic and technical sources of variation in SSR data. – Mol. Breed. 10: 181–191.

Helentjaris, T., Weber, D. and Wrights, S. 1988. Identification of genetic locations of duplicate nucleotide sequence in maize by analysis of restriction fragment length polymorphisms. – Genetic 118: 353–363.

Hintze, J. L. 1998. STATISTICAL system for Windows. Number Chuncher Statistical Systems Kayville, Utah.

Jiang, C., Edmeades, G. O., Armstead, I. et al. 1999. Genetic analysis of adaptation differences between highland and lowland tropical maize using molecular markers. – Theor. Appl. Genet. 99: 1106–1119.

Kubik, C., Sawkins, M., Meyer, W. A. et al. 2001. Genetic diversity in seven perennial ryegrass (*Lolium perenne* L.) cultivars based on SSR markers. – Crop Sci. 41: 1565–1572.

Liu, Z. W., Biyashev, R. and Saghai-Maroof, M. 1996. Development of simple sequence repeats DNA markers and their integration into a barley linkage map. – Theor. Appl. Genet. 93: 869–876.

Liu, K., Goodman, M., Muse, S. et al. 2003. Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites. – Genetics 165: 2117–2128.

Matsuoka, Y., Mitchell, S. E. and Kresovich, S. 2002. Microsatellites in *Zea* – variability, patterns of mutations and use for evolutionary studies. – Theor. Appl. Genet. 104: 436–450.

Pejic, I., Ajmon-Marson, P., Morgante, M. et al. 1998. Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, and AFLPs. – Theor. Appl. Genet. 97: 1248–1255.

Pinto, L. R., Vieira, M. L. C., de Souza, C. L. et al. 2003. Genetic diversity assessed by microsatellites in tropical maize population submitted to high-density reciprocal recurrent selection. – Euphytica 134: 277–286.

Pixley, K. V. and Zambezi, B. T. 1996. Maize germplasm available from CIMMYT-Zimbabwe. – CIMMYT, Harare, Zimbabwe.

Powell, W., Morgante, M., McDevitt, R. et al. 1995. Polymorphic simple sequences repeat regions in chloroplast genomes: application on population genetics of pines. – Proc. Natl Acad. Sci. USA 92: 7759–7763.

Reif, J. C., Melchinger, A. E., Xai, X. C. et al. 2003. Use of SSRs for establishing heterotic groups in subtropical maize. – Theor. Appl. Genet. 107: 947–957.

Schechert, A. W., Welz, H. G. and Geiger, H. H. 1999. QTL for resistance to *Setophaeria turcica* in tropical African maize. – Crop Sci. 39: 523–524.

Schug, M. D., Hutter, C. M., Wetterstr, K. A. et al. 1998. The mutation rate of di, tri and tetra-nucleotide repeats in *Drosophila melanogaster*. – Mol. Biol. Evol. 15: 1751–1760.

Senior, M. L., Chin, E. C. L., Lee, M. et al. 1996. Simple sequences repeat markers developed from maize sequences found in the GENEBANK database: map construction. – Crop Sci. 36: 1676–1683.

Senior, M. L., Murphy, J. P., Goodman, M. M. et al. 1998. Utility of SSRs for determining genetic similarities and relationships in maize using agarose gel system. – Crop Sci. 38: 1088–1098.

Sibov, S. T., de Souz Jr, C. L., Garica, A. A. F. et al. 2003. Molecular mapping in tropical maize (*Zea mays* L.) using microsatellite markers. 1. Map construction and localization of loci showing distorted segregation. – Hereditas 139: 96–106.

Smith, J. S. C. and Smith, O. S. 1992. Fingerprinting crop varieties. – Rev. Agric. Acad. Press, USA, p. 85–140.

Smith, J. S. C., Chin, E. C. L., Shu, H. et al. 1997. An evaluation the utility of SSR loci as molecular markers in

maize (*Zea mays* L.): comparison with data from RFLP and pedigree. – Theor. Appl. Genet. 95: 163–173.

Taramino, G. and Tingey, S. 1996. Simple sequence repeats for germplasm analysis and mapping in maize. – Genome 39: 277–287.

Tautz, D. 1989. Hypervariablity of simple sequences as a general source of polymorphic DNA markers. – Nucleic Acids Res. 17: 6463–6471.

Vaz Patto, M. C., Satovic, Z., Pêgo, S. et al. 2004. Assessing the genetic diversity of Portuguese maize germplasm using microsatellite markers. – Euphytica 137: 63–72.

Warburton, M. L., Xianchun, X., Crossa, J. et al. 2002. Genetic characterization of CIMMYT inbred maize lines and open pollinated populations using large scale fingerprinting methods. – Crop Sci. 42: 1832–1840.

Weber, J. L. 1990. Informativeness of human $(dC - dA)_n$. $(dG - dT)_n$ polymorphisms. – Genomics 7: 524–530.

Weising, K., Winter, P., Huttel, P. B. et al. 1998. Microsatellite markers for molecular breeding. – J. Crop Prod. 1: 113–143.

Welz, H. G., Schechert, A. W. and Geiger, H. H. 1999. Dynamic gene action at QTLs for resistance to *Setosphaeria turcica maize*. – Theor. Appl. Genet. 98: 1036–1045.

Westman, A. L. and Kresovich, S. 1997. Use of molecular marker techniques for description of plant genetic variation. – In: Callow, J. L., Ford-Lloyd, B. V. and and. Newburry, H. J (eds), Biotechnology and plant genetic resources. CAB Int, p. 9–45.

Yuan, L. X., Fu, J. H., Warburton, M. L. et al. 2000. Comparison of genetic diversity among maize inbred lines based on RFLPs, SSRs, AFLPs and RAPDs. – Acta Genet. Sin. 27: 725–733.