Genetic Diversity of Maize (*Zea mays* L.) Inbreds Developed for Highlands and Mid-Altitudes as Revealed by AFLP Markers

Legesse Wolde[1], A.A. Myburg[2], K. Pixley[3] and A.M. Botha[2]

[1]Ethiopian Institute of Agricultural Research, P.O. Box 2003, Addis Ababa, Ethiopia; [2]Department of Genetics & Forestry and Agricultural Biotechnology Institute,, University of Pretoria, 0002, Pretoria, South Africa; [3]CIMMYT, Int. Col, El Batan, Texcoco Edo. De Mexico, C.P. 56130, Mexico

Abstract

Fifty six maize inbred lines developed for highlands and mid-altitudes of Ethiopia and Zimbabwe were fingerprinted using amplified fragment length polymorphism (AFLP) markers. The objectives were to investigate the genetic relationships among the inbred lines and to cluster them into heterotic groups with a view to generate broad-based breeding populations. Seven pre-screened pairs of AFLP primers identified 499 scorable fragments, of which 408 (81.7%) were polymorphic. The genetic diversity (Euclidean distance) varied from 0.35 to 0.71 with an average of 0.58. The UPGMA clustering using average linkage methods distinguished four major groups. The highland inbred lines generally separated from the mid-altitude germplasm. Each of these groupings possessed a number of subclusters mostly related to the pedigree records of the inbred lines. Principal coordinate analysis, also demonstrated considerable genetic divergence between and within the genotypes of the different origins. This groupings based on AFLP markers can be utilized to generate heterotic populations that can serve as source material to develop superior inbred lines revealing good combining ability. Moreover, inbred lines of different heterotic groups can be used to launch crossing activities leading to the development of high yielding maize hybrid and synthetic varieties.

Key words: Amplified fragment length polymorphism, genetic diversity, heterotic groups, inbred lines, maize, *Zea mays* L.

Introduction

In Ethiopia, maize (*Zea mays* L.) is an important cereal in terms of acreage, total production and utilization. It grows in the highlands, mid-altitudes and lowlands including drought prone areas. Among cereals, maize stands first in productivity and total production, and second to tef [*Eragrostis tef* (Zucc.) Trotter] in acreage (CSA, 2004). Maize covers approximately 20% of the cultivated area in the highlands where about 30% of small-scale farmers depend on it for their living (EARO, 2000). However, maize varieties mostly grown in the highlands of Ethiopia are local cultivars with poor agronomic performance and low average yield (Twumasi-Afriyie *et al*., 2002). Also diseases such as gray leaf spot (*Cercospora zeae-maydis* Tehon), northern leaf blight (*Exserohilum turcicum)* and leaf rust *(Puccina sorgi)* are major limitations. The national maize breeding program is lacking broad-based genetic materials for the development of improved maize varieties.

Success in the development of improved maize varieties depends on the identification and use of different genetic materials to form broad-based source germplasm. Using conventional methods, some works have been conducted to identify genetic diversity among certain inbred lines (Bayisa *et al*., 2008; Legesse *et al.,* 2009). However, maize breeding is a continuous process and requires a wide

range of diverse and accuarately identified and sorted source germplasm materials using molecular markers.

Currently, several molecular marker techniques are available and used for various purposes in crops. AFLP is one of the molecular marker systems relying on polymerase chain reaction (PCR) techniques (Mullis *et al*., 1986) for DNA amplification. It possesses a number of attractive features relative to other markers including (i) a high multiplex ratio, (ii) ability to give highly reproducible banding patterns, and (iii) requirement of limited number of generic primers (Pejic *et al*., 1998). Furthermore, AFLP requires no DNA sequence information and can detect large number of genetic loci than restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers (Pejic *et al*., 1998; Bohn *et al*., 1999; Lübberstedt *et al*., 2000). On the other hand, AFLP is less sensitive in DNA scoring potential than single nucleotide polymorphic (SNP) markers. However, SNPs are expensive and demand high technology input. In addition, special instruments are needed for SNPs genotyping (Ching *et al*., 2002; Soleimani *et al*., 2003). In contrast, AFLP techniques are less costly and are more transferable across species than SSR markers (Garecia *et al*., 2004).

AFLP, on the bases of its attractive features, has gained considerable acceptance for DNA fingerprinting of wide range of crop species including maize (Melchinger, 1999). In maize several studies have demonstrated the robustness of AFLPs for assessing genetic similarity of maize germplasm that are consistent with their pedigree relatedness and with unknown or uncertain genetic background (Garecia *et al.,*2004; Li *et al*., 2005). Moreover, AFLPs have been found to heterotically group inbred lines with maximum reliance by revealing pedigree relationships (Lübberstedt *et al*., 2000). In addition, AFLPs have been used for construction of genomic maps, (Ajmone-Marsan *et al.,* 2001) DNA, fingerprinting (Garecia *et al*., 2004), and prediction of hybrid performances (Legesse *et al*., 2007).

The objectives of the present study were to investigate the extent and pattern of genetic relationships among highland and mid-altitude adapted maize inbred lines in Ethiopia, and to assign them into the different heterotic groups using AFLP markers.

Materials and Methods

Plant materials

Fifty-six maize inbred lines adapted to the mid-altitude and highland environments in Ethiopia and Zimbabwe were included in this study (Table 1). Among these, 21 0f the inbred lines are of mid-altitude materials received from CIMMYT Zimbabwe. These inbred lines are of miscellaneous origin and possess tolerance or resistance against maize streak virus, gray leaf spot and northern leaf blight (Pixley and Zambezi, 1996; Schechert *et al*., 1999; Welz *et al.,* 1999). Three of the inbred lines are of local origin, and are known to be successful line testers in Ethiopia. The remaining 32 inbred lines were introductions through the CIMMYT-Ethiopia Highland Maize Research Project, and were selected for adaptation in the highlands.

DNA isolation

Leaf tissue from each inbred line was harvested from three- to four-week-old seedlings grown in the greenhouse. Genomic DNA was isolated using the Hexa-decyltrimethyl-ammonium bromide (CTAB) DNA extraction procedure (Doyle and Doyle, 1987). Approximately, 50 mg of leaf tissues from each inbred line was

sampled into a 2 ml screw-cap tube containing 800 µl of 5% (w/v) CTAB extraction buffer for high-throughput AFLP analysis using LI-COR IR^2 Automated Sequencers (Myburg *et al.,* 2001). The leaf tissues were ground into a fine paste using a FastPrep FP120 Instrument (QBiogene, Carlsbad, CA, USA) and later incubated at 65 °C for 60 minutes. The samples were extracted once with chloroform iso-amyl alcohol (24:1) and washed 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, 0.2 mM EDTA), and the DNA concentration was estimated by comparison to a serial dilution of lambda DNA standard on a 1% agarose gel.

AFLP analysis

AFLP analysis was performed according to the protocol of Vos *et al*., (1995) with the modification that 250 ng of DNA were digested at 37 °C for 2 h using 6 U and 4 U *Eco*RI and *Mse*I restriction endonucleases, respectively. Digested products were tested for their consistency, and ligation was made using oligonucleotide adapters specific for the *Eco*RI and *Mse*I cleavage sites. All ligations were incubated overnight at 37ºC. The samples were then analysed on a 1% agarose gel with 100 bp to check for ligation occurrence. Following adapter ligation, pre-selective and selective amplification reactions were performed.

The preselective amplification reactions were performed with standard *Eco*RI (E+A) and *Mse*I (M+C) (Vos *et al.,* 1995) in 35 µl volume containing 10 µl of 40-fold diluted ligation product, AFLP pre-amplification primer mix (10 µM *Eco*RI and 10 µM *Mse*I primers, 2.5 mM dNTPs each), 10X PCR buffer, 1.5 mM $MgCl_2$, 1U Taq DNA polymerase (Promega). The following thermocycling profile was used for selective preapmplification for 30 cycles: adapter extension for 10 s at 72 °C followed by denaturation for 10 s at 94 °C, annealing for 30 s at 56 °C, and extension for 1 min at 72 °C with a 1 s per cycle increase in extension time per cycle. The pre-amplification products were tested for amplification on a 1% agarose gel.

The final selective amplifications were performed in all the inbred lines using seven selective AFLP primer combinations, which were chosen based on pre screening of a sample of eight inbred lines with 24 selective primers which were used to ascertain the extent of polymorphism. Standard EcoRI (E +ANN) and MseI M +CNN) adapter-primers with three selective nucleotides were used for the final analysis (Table 2). *Eco*RI primers were 5'-labeled with infrared dye (IRDye 700 or IRDye 800, LI-COR, Lincoln, NE, USA). The reactions were performed in 11 µl volume of final concentration containing 5 µl diluted (X10) pre-amplification reaction, 10X PCR buffer (1.5 mM $MgCl_2$), 2.5 mM of each dNTPs, 0.5 mM additional $MgCl_2$, 1 µM IRDye 700/800-labeled *Eco*RI primers, 10 µM *Mse*I primer, and 5U Ampli-Taq DNA polymerase (Promega). AFLP fragments were resolved on 8% Long Ranger™ polyacrylamide gels using the LI-COR IR^2 Automated DNA Analyser (LI-COR Lincoln, NE, USA). The presence and absence of band was scored as 1 and 0, respectively.

Table 1. Identification and pedigrees of highland and mid-altitude adapted maize inbred lines

Identification	Pedigree	Parents/ Source	Adaptation	Origin
Amboon6-1	KIT/SNSYN ((N3) TUXC1F1 # # # (GLS=1) 6-1	Kitale Syn II	H.land	CIMMYT
Amboon6-3	KIT/SNSYN ((N3) TUXC1F1 # # # (GLS=1) 6-3	Kitale Syn II	H.land	CIMMYT
Amboon6-4	KIT/SNSYN ((N3) TUXC1F1 # # # (GLS=1) 14-1	Kitale Syn II	H.land	CIMMYT
Amboon6-6	KIT/SNSYN ((N3) TUXC1F1 # # # (GLS=1.5) 7-3	Kitale Syn II	H.land	CIMMYT
Amboon6-8	KIT/SNSYN ((N3) TUXC1F1 # # # (GLS=1) 7-3	Kitale Syn II	H.land	CIMMYT
Amboon6-9	KIT/SNSYN ((N3) TUXC1F1 # # # (GLS=1) 11-1	Kitale Syn II	H.land	CIMMYT
Amboon6-10	KIT/SNSYN ((N3) TUXC1F1 # # # (GLS=1) 33-2	Kitale Syn II	H.land	CIMMYT
Amboon6-14	KT/SNSYN ((N3) TUXC1F1 # # (GLS=1) 11-2	Kitale Syn II	H.land	CIMMYT
Amboon6-15	KIT/SNSYN ((N3) TUXC1F1 # # # (GLS=1) 14-2	Kitale Syn II	H.land	CIMMYT
Amboon6-20	SRSYN95 ((KIT/N3) TUXF1 # # # (GLS=1) 6-1	Kitale Syn II	H.land	CIMMYT
Amboon6-21	ECU/SNSYN (SC/ETO) C1 F1 # ## (GLS=1.5) 16-1	Ecuador 573	H.land	CIMMYT
Amboon6-22	ECU/SNSYN (SC/ETO) C1 F1 # ## (GLS=2.0)-3-1	Ecuador 573	H.land	CIMMYT
Amboon6-23	ECU/SNSYN (SC/ETO) C1 F1 # ## (GLS=2.0)-8-2	Ecuador 573	H.land	CIMMYT
Amboon6-25	ECU/SNSYN (SC/ETO) C1 F1 # ## (GLS=2.5)-24-2	Ecuador 573	H.land	CIMMYT
Amboon6-26	ECU/SNSYN (SC/ETO) C1 F1 # ## (GLS=2.5)-42-3	Ecuador 573	H.land	CIMMYT
Amboon6-27	ECU/SNSYN (SC/ETO) C1 F1 # ## (GLS=3.0)-23-1	Ecuador 573	H.land	CIMMYT
Amboon6-29	ECU/SNSYN (SC/ETO) C1 F1 # ## (GLS=3.5)-41-1	Ecuador 573	H.land	CIMMYT
Amnoon6-34	SRSYN95 ((ECU/SC/ETO) F1 # # # (GLS=2)-18-2	Ecuador 573	H.land	CIMMYT
Amboon6-37	SRSYN95 ((ECU/SC/ETO) F1 # # # (GLS=3)-21-1	Ecuador 573	H.land	CIMMYT
Amboon6-38	SRSYN95 ((ECU/SC/ETO) F1 # # # (GLS=3.5)-40-1	Ecuador 573	H.land	CIMMYT
Amboon6-39	SRSYN95 ((ECU/SC/ETO) F1 # # # (GLS=3.5)-4-2	Ecuador 573	H.land	CIMMYT
Amboon6-40	SRSYN95 ((ECU/SC/ETO) F1 # # # (GLS=3.5)-39.1	Ecuador 573	H.land	CIMMYT
Amboon6-41	POOL9AC-7-SR (BC2) FS-1-1-3-1	Pool9A	H.land	CIMMYT
Amboon6-42	POOL9AC-7-SR (BC2) FS-1-4-2-3	Pool9A	H.land	CIMMYT
Amboon6-44	POOL9AC-7-SR (BC2) FS-4-3-SR-1-1	Pool9A	H.land	CIMMYT
Amboon6-47	POOL9AC-7-SR (BC2) FS-50-1-2-3	Pool9A	H.land	CIMMYT
Amboon6-49	POOL9AC-7-SR (BC2) FS-89-2SR-1-1	Pool9A	H.land	CIMMYT
Amboon6-51	POOL9AC-7-SR (BC2) FS-123-2-1-3	Pool9A	H.land	CIMMYT
Amboon6-54	POOL9AC-7-SR (BC2) FS-170-4-1-3	Pool9A	H.land	CIMMYT
Amboon6-58	POOL9AC-7-SR (BC2) FS-222-4-1-3	Pool9A	H.land	CIMMYT
Amboon6-59	POOL9AC-7-SR (BC2) FS-232-4-1-3	Pool9A	H.land	CIMMYT
Amboon6-60	POOL9AC-7-SR (BC2) FS-48-1-1-3	Pool9A	H.land	CIMMYT
142 –1-e	Unknown (derived from Ecuador 573 in Ethiopia)		M.altitude	Ethiopia
F7215	Unknown (derived from Kitale Synetic II in Ethiopia)		M.altitude	Ethiopia
POOL 9A-MHM	Unknown (derived from pool9A in Ethiopia)		H.land	Ethiopia
CML202	ZSR923-S4BULK-5-1-BBB		M.altitude	CIMMYT
CML204	[7794]-SELF-4-1-S9-1-4-7-4-5-BBB		M.altitude	CIMMYT
CML206	[EV7992#/EVPOP44-SRBC3]#BF37SR-2-3SR-2-4-3-BBB		M.altitude	CIMMYT
CML216	[MSR:131]-3-3-3-5-BBB		M.altitude	CIMMYT
CML312	[S89500F2-2-2-1-1-B*5		M.altitude	CIMMYT
CML386	[EV7992#/EVPOP43-SRBC3]#b#bsr-118-2-2-5-7-B-1-1-B*4	ZM601	M.altitude	CIMMYT
CML387	[EV7992#/EV8449-SR] C1F2-334-1(OSU8i)-1-1-X-X-3-BB	ZM605	M.altitude	CIMMYT
CML388	[EV7992#/EV8449-SR] C1F2-334-1(OSU9i)-8-2(I)-X-1-2-BB	ZM605	M.altitude	CIMMYT
CML389	[EV7992#/EV8449-SR]C1F2-334-1(OSU9i)-8-6(I)-X-X-3-BB	ZM605	M.altitude	CIMMYT
CML390	[EV7992] C1F2-430-3-3-3-X-7-BB	EV7992	M.altitude	CIMMYT
CML391	[EV7992] C1F2-430-3-3-X-1-BB	EV7992	M.altitude	CIMMYT
CML392	[M37W/100MSR//SR52/ZAMXSR7794-4-3]#B-111-1-5-B*5		M.altitude	CIMMYT
CML393	[R201/TZMSRW]#B-18-1-1-3-2-X-1-BB		M.altitude	CIMMYT
CML394	[PL31/POOL16SR//PL9A] C1F2-124-2-X-X-X-BB		M.altitude	CIMMYT
CML395	90323(B)-1-X-1-B-B-1-1-B-B-1-1-B		M.altitude	CIMMYT
CML440	G16SeqC1F47-2-1-2-1-BBBBB	G16	M.altitude	CIMMYT
CML441	ZM605C2F1-17-1-B-1-BB	ZM605	M.altitude	CIMMYT
CML442	M37W/ZM607#bF37sr-2-3sr-6-2-X]-8-2-X-1-BBBB		M.altitude	CIMMYT
CML443	[AC8342/IKENNE {1} 8149SR//PL9A] C1F1-500-4-X-1-1-BB-1BB	ZM605	M.altitude	CIMMYT
CML444	P43C9-1-1-1-1-1-BBBBB	P43	M.altitude	CIMMYT
CML445	[[TUXPSEQ] C1F2/P49-SR]F2-45-7-5-1-B		M.altitude	CIMMYT

Data analysis

The average polymorphism information content (PIC) was calculated across each primer combination according to Riek *et al*., (2001) as follows:

$$PIC = 1 - \left[f^2 + \left(1 - f \right)^2 \right]$$

where f is the frequency of the marker bands in the data set.

Marker index (MI) was determined by multiplying PIC values with percentage polymorphism for each primer combination (Lübberstedt *et al*., 2000). Estimation of similarity among all pairs of the lines were made from the data matrices in the form of dissimilarity coefficients and expressed as Euclidean genetic distance (Hintze, 1998). Cluster analysis was performed to generate a dendrogram using the Unweighted Pair Group Method based on Arithmetic averages (UPGMA) as used in the NCSS software package. (Hintze, 1998). Principal coordinate analysis (PCoA) was performed using the genetic dissimilarity matrix to explore the relationship of the inbred lines on a two-dimensional plot (Hintze, 1998). The "goodness of fit" of the clustering algorithm to the data matrix was determined by calculating the cophenetic correlation coefficient between the dissimilarity matrix and the cophenetic matrix derived from the dendrogram (Sneath and Sokal, 1973**).**

Results and Discussion

AFLP polymorphism

A total of 499 AFLP bands with sizes ranging from 54 to 725 bp were scored for seven selective AFLP primer combinations across the inbred lines. The number of polymorphic bands detected after selective amplification was 409 and varied from 49 to 58. Polymorphism across the inbred lines for all selective primer combinations varied from 79.7% to 82.7%. Polymorphic information content (PIC) and marker index (MI) values for each of the primer pairs ranged from 0.29 to 0.37 and 15.4 to 24.1, with mean of 0.3 and 19.2, respectively (Table 2).

These results are consistent with previous findings, thus, supporting that AFLP marker system can be efficiently utilized in genetic diversity studies (Lübberstedt *et al*., 2000; Oliviera *et al*., 2004; Li *et al*., 2005). In addition, the assay efficiency index results of the study are in agreement

Table 2. Sequence ID of EcoRI and MseI primers, number of scored bands, and estimates of degree of polymorphism, polymorphic information content (PIC) and marker indices (MI) of 56 maize inbred lines using AFLP markers

Seq.ID of *Eco*RI Primers[a]	Seq.ID. MseI Primers	No. of scored Bands	No. of polymorphic Bands	Polymorphism (%)	PIC	MI
E32-AAC(800)	M57-CGG	65	53	81.5	0.29.	15.4
E35-ACA (700)	M48-CAC	73	60	82.2	0.33	19.8.
E35-ACA(700)	M52-CCC	83	68	81.9	0.32	21.8
E35-ACA (700)	M61-CTG	74	59	79.7	0.37	21.8
E37-ACG/ (700)	M53-CCG	63	52	82.5	0.31	16.1
E41-AGG (800)	M48-CAC	81	67	82.7	0.36	24.1
E41-AGG(800)	M49-CAG	60	49	81.6	0.35	17.2
Mean		71.2	58.2	81.7	0.33	19.2
Total		499	408			

[a] Sequence ID of **s**elective nucleotides of *Eco*RI/*Mse*I adapter primers and IRD 700 or IRD 800 labelled primers

with other findings (Pejic *et al*., 1998, Bohn *et al*., 1999; Lübberstedt *et al*., 2000) that showed the effectiveness of AFLP markers in detecting large number of polymorphism as compared to other marker systems, and pointed out that AFLP can replace RFLP, RAPD and SSR markers in genetic diversity studies because of its comparable accuracy in genotyping inbred lines of diverse origins.

Relationships among the inbred lines

The scorable data obtained from the seven selective primer combinations were used to determine the genetic similarity among the 56 maize inbred lines using dissimilarity coefficients. The dissimilarity matrices were then used in the cluster analysis to generate a dendrogram using UPGMA method.

The genetic dissimilarity estimates ranged from 35% to 71% with overall mean of 58% (Fig.1.). The highest genetic dissimilarity (71%) was between inbred lines Ambo6-8 and 142-1-e, while the lowest genetic dissimilarity (35%) was exhibited between CML388 and CML389. The minimum genetic distance (0.35) revealed between CML388 and CML 389 is to be expected because these inbred lines share a common parent as indicated in their pedigree records. Similarly, the maximum genetic distance (0.71) manifested between Ambo6-8 and 142-1-e, reflects their clear divergence in their origin since inbred line Ambo6-8 was derived from Kitale Syn.II and inbred 142-1-e traces towards Ecuador 573. Kitale Syn.II and Ecuador 573 possesses a broad range of divergence in terms of their genetic composition and hence are well kown heterotic populations under Eastern African conditions (Darrah, 1986).

The genetic relationships were expressed as shown in the dendrogram (Fig.1) illustrating the graphical representation of the genetic distance among the 56 maize inbred lines. The dendrogram has a relatively high cophenetic coefficient (0.86) and thus, shows a very good fit with the genetic distance values (Sneath and Sokal, 1973). The UPGMA clustering grouped the inbred lines into four main clusters. Cluster I was composed of the three inbred line testers. In Cluster II, mid-altitude inbred lines obtained from CIMMYT were grouped with a number of sub-clusters included within the major group. Cluster III contained highland maize inbred lines as a major group. This was further sub-divided into three clusters (Kitale Syn II, Ecuador-573 and Pool9A groups) mainly based on pedigree origin. Cluster IV was composed of mixtures of highland and mid-altitude inbred lines.

With the exception of cluster I, the inbred lines grouped in each of the clusters were in most cases consistent with their pedigree. Principal coordinate analysis has also confirmed the aforementioned groupings mostly based on their pedigree information. The results are generally congruent with the findings of previous studies on genetic diversity analysis and heterotic groupings of maize inbred lines using molecular markers (Smith and Smith, 1992; Dubreuil and Charcosset, 1999; Lübberstedt *et al*., 2000; Warburton *et al*., 2002; Oliveira *et al*., 2004). These investigators concluded that inbred lines sharing common ancestors tend to cluster together, thereby suggesting that the genetic similarity values obtained by molecular markers appear to correspond to the known pedigree information. However, certain violations as revealed by the inbred lines in Cluster IV have been observed. Such instances are not uncommon when comparing molecular results with classification based on pedigree information. Effects of selection, genetic drift and mutation on the DNA markers or human error might be the cause for the differences detected (Warburton *et al*., 2002)

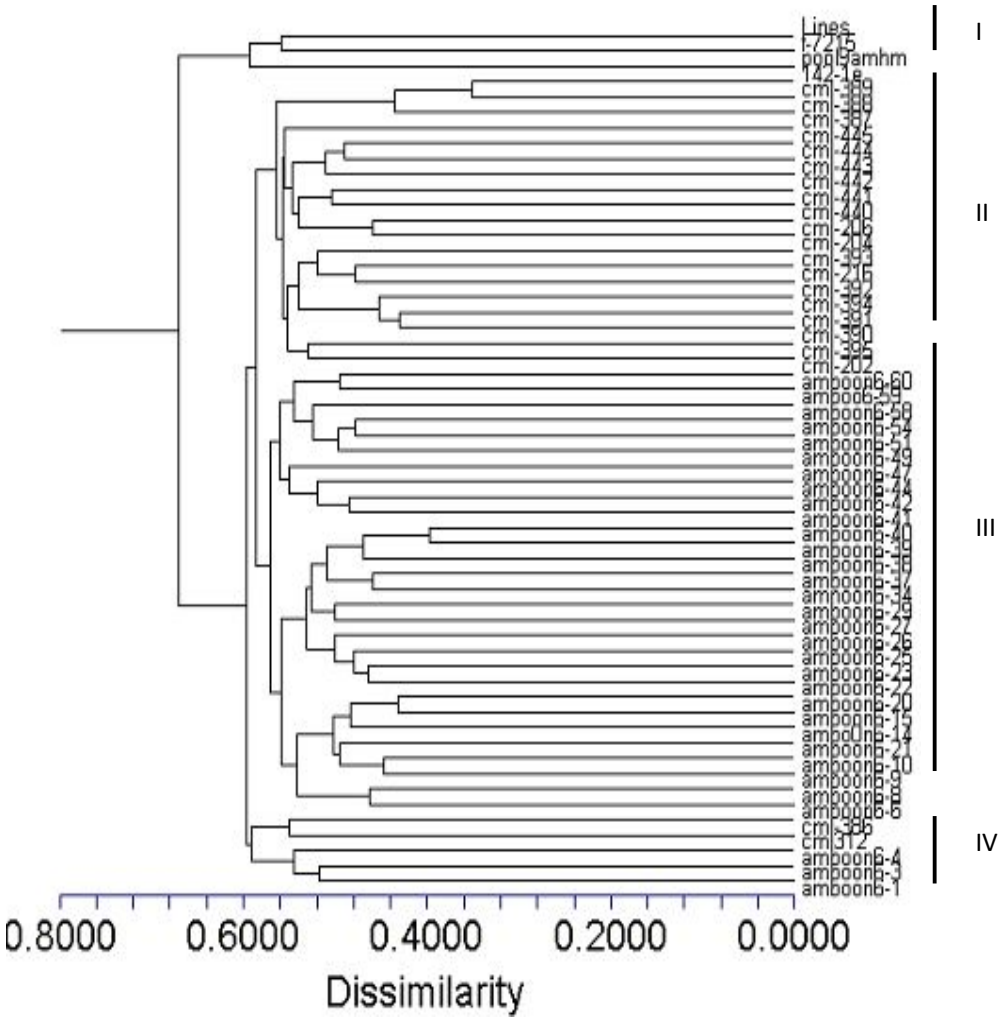

Fig. 1. Dendrogram showing relationships among 36 maize inbred lines based on UPGMA clustering using dissimilarity coefficients of AFLP markers (Groups I, II, III and IV indicate major clusters)

The relationship among the 56 maize inbred lines revealed by PCoA calculated from AFLP genetic distance estimates accounted for 13.2% and 9.4% of the total variation in the first and second coordinates, respectively (Fig. 2). The analysis positioned highland inbred lines distinctly from mid-altitude lines. Among the highlands inbred lines there were distinct sub-grouping as reflected by their pedigree. That about 22% of the total variability among the inbred lines was explained by the two principal coordinates is in close agreement with the results of previous studies in maize inbred lines using RFLP (Livini *et al*., 1992) and AFLP (Lübberstedt *et al*., 2000) markers.

In this study, the grouping of the inbred lines mostly reflected their pedigree relatedness. However, distinction among the inbred lines based on their adaptation regime (highland and mid-altitude), have been observed (Cluster II & III) in the cluster analysis. It is not clear whether or not polymorphism due to differences in adaptation regime might have influenced the phenomenon. In a study conducted to investigate the genetic base for adaptation differences between highland and lowland tropical maize based on RFLP markers

Jiang *et al*. (1999) indicated the relative importance of genomic segments and their effects on adaptation to thermally diverse locations. Hence, based on their findings, we may speculate the role of genes specific for adaptation regime to account for some level of variation between the highland and mid-altitude sources of the inbred lines.

Another interesting feature in our study is the separation of inbred lines of common ancestry (mid-altitude and highland). These inbred lines, which have been depicted of highland and mid altitude adaptations share common ancestors; however, cluster analysis and also principal coordinate analysis separated the lines into distinct groups. A number of explanations could be given for this mainly on the basis of historical perspectives of the inbred lines. Basically, the development of the inbred lines differs in time and space. The ancestral populations, although they are similar by denomination, have been maintained by different breeding programs. Therefore, difference in origin, duration of development and methods of germplasm maintenance (Gethi *et al*., 2002) could have contributed to the occurrence of this variation. This speculation is in line with the aspects of changes in genetic materials with time due to natural and artificial selection, and genetic drift (Ajmone-Marsan *et al*., 2001; Senior *et al*., 1998). Moreover, with change in geographical distance germplasm may attain certain peculiar characteristics, which make them genetically different from other corresponding germplasm in different locations (Moll *et al*., 1965)**.** Generally, cluster and principal coordinate analysis based on AFLP data separated most of the inbred lines into different groups in a manner that is largely consistent with their pedigree records. Between inbred lines of common ancestors but of different adaptation regimes marked differences in their grouping was observed, which may be attributed to geographic distance, drift, selection or human error (Moll *et al*., 1965; Gethi *et al*., 2002).

Fig. 2. Associations among 56 maze inbred lines revealed by principal coordinate analysis performed on genetic dissimilarity estimates calculated from the AFLP data.

Overall, the present results clearly described the usefulness of the AFlP marker system for fingerprinting maize inbred lines with high accuracy. This could be attributed to the high multiplex ratio of the AFLP assay. The markers separated the inbred lines into different clusters mainly based on pedigree records indicating the robustness of AFLP markers for diversity analysis and heterotic groupings. Accordingly, our findings showed that the majority of the pairwise combinations exhibited more than 58% variation suggesting ample opportunity to utilize the germplasm as source material to establish heterotic populations and to identify promising hybrid combinations.

Acknowledgements

The study was conducted at the University of Pretoria, South Africa, and was funded by the Ethiopian Institute of Agricultural Research.

References

Ajmone-Marsan, P. Gorni, Chitto, C. Redaelli, R. van Vijk, R. Stam, P. and M. Motto. 2001. Identification of QTL for grain yield and grain related traits of maize (*Zea mays* L.) using an AFLP map, different testers and cofactor analysis. Theor. Appl. Genet. 102: 230-243.

Bayisa, A., Hussein, M. and Z. Habtamu, 2008. Combining ability of transitional highland maize inbred lines. East Afr. J. Sci. 2:19-24.

Bohn, M., Utz, H.F. and A.E. Melchinger, 1999. Genetic similarities among winter wheat cultivars determined on the bases of RFLPs, AFLPs, and SSRs and their use for predicting progeny variance. Crop Sci. 39: 228-257.

Ching, A.D.A., and A. Rafalski, 2002. Rapid genetic mapping of ESTs using SNP pyrosequence and idle analysis. Cell. Mol. Biol. Lett. 7:803-810.

CSA. 2004. Central Statistical Authority Cultivated area and yield of major crops in Ethiopia. Addis Ababa, Ethiopia

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

Dubreuil, P., and A. Charcosset. 1999. Relationships among maize inbred lines and populations from European and North American germplasm as estimated using RFLP markers. Theor. Appl. Genet. 99: 473-480

EARO. 2000. Research Strategy for Maize. Ethiopian Agricultural Research Organization (EARO), Addis Ababa, Ethiopia.

Garecia, A.A.F., Benchimol, L.L., Barbosa A.A.M., Geraldi, I.O. Souza, C.L.Jr. and A.P. de Souza. 2004. Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. Genet. Mol. Biol. 27: 279-288.

Gethi, J.G., Labate, J.A., Lamkey, K.R. Smith, M.E. and S. Kresovich. 2002. SSR variation in important maize inbred lines. Crop Sci. 42:951-957.

Hintze, J.L., 1998. NCSS 2000. Statistical System for Windows. Number Cruncher Statistical Systems. Kaysville, Utah.

Jiang, C., Edmeades, G.O. Armstead, I. Lafitte, H.R., Hayward, M.D. and D. Hoisington. 1999. Genetic analysis of adaptation differences between highland and lowland tropical maize using molecular markers. Theor. Appl. Genet. 99:1106-1119.

Lee, M. 1995. DNA markers and plant breeding programs. Adv. Agron. 55: 265-344

Legesse, B.W., Myburg, A.A. Pixely, K.V. Twumasi-Afriyie, S. and A.M. Botha. 2007. Relationship between hybrid performance and AFLP based genetic distance in highland maize inbred lines. Euphytica 162:313-323.

Legesse, B.W., Myburg, A.A., Pixely, K.V. and A.M. Botha. 2009.

Combining ability and heterotic grouping of highland transition maize inbred lines. Maydica 54: 1-9.

Li, Y., Shi, Y.S., Song, Y.C. Du, J.Y., Tuberosa, R. and T.Y. Wang. 2005. Analysis of genetic diversity in maize inbred lines based on AFLP markers. Maydica 49:89-95.

Livini, C., P. Ajmone-Marson, A.E. Melchinger, M.M. Messmer, and M. Motto. 1992. Genetic diversity of maize inbred lines within and among heterotic groups revealed by RFLPs. Theor. Appl. Genet. 64: 17-25.

Lübberstedt, T.,. Melchinger A.E., Du, C., le Vuylsteke, M. and M. Kuiper. 2000. Relationships among early European maize inbreds: IV. Genetic diversity revealed with AFLP markers and comparison with RFLP, RAPD, and pedigree data. Crop Sci. 40: 783-791.

Melchinger, A.E. 1999. Genetic diversity and heterosis, pp 99-113. In: Coors, J.G., and S. Pandey. (eds.). Genetic and exploitation of heterosis in crops, A. Soc. of Agronomy, Crop. Sci. Soc. America, Soil Sci. Soc. of America, Madison, Wisconsin, USA.

Moll, R.H., Lonnquist, J.H. Furtuna, J.V. and E.C. Johnson. 1965. The relationship of heterosis and Genetic divergence in maize. Genetics 52: 139-144.

Mullis, K., Faloona, F., Saike, R., Horn, G. and H. Erlich. 1986 Enzymatic amplification of DNA *in vitro*: the polymerase chain reaction. Cold Spring Harbor Symp. Quant Biol. 51: 261-273.

Myburg, A.A., Remington, D.M., Õ Malley, R.R., Sederoff, and R.W. Whetter. 2001. High-throughput AFLP analysis using inferared dye-labeled primers and an automated DNA sequencer. Bio Techniques 30:348-357.

Oliveira, K.M., Laborda, R.P.,. Garcia, A.A.F.. Paterniani, M.E.A.G.Z. and A.P. de Souza, 2004. Evaluating genetic relationships between tropical maize inbred lines by means of AFLP profiling. Hereditas 140:24-33.

Pejic, I., P. Ajmone-Marsan, Morgante, M. Kozumplick, V. Castiglioni, P. Tarmino, G. and M. Motto. 1998. Comparative analysis of genetic similarity among maize inbred line detected by RFLPs, RAPDs, and AFLPs. Theor. Appl. Genet. 97:1248-255.

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

Riek, J., Calsyn, E. Everaet, I. van Bockstaele, E. and M. Loose, 2001. AFLP based alternative for the assessment of distinctness, uniformity and stability of beet varieties. Theor. Appl. Genet. 103:1254 -1265.

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

Senior M.L., Murphy, J.K. Goodman, M.M. and C.W. Stuber, 1998. Utility of SSR for determining genetic similarities and relationships in maize using agarose gel system. Crop Sci. 38: 1088-1098.

Sheng, W.M., and D.J. Rui. 2000. Use of AFLP markers to predict hybrid yield and yield heterosis in maize. Acta-Bot. Sci. 42: 600-604.

Smith, J. S. C., and O.S. Smith. 1992. Fingerprinting crop varieties, pp. 85-140. Rev. of Agr. Academic press, USA

Sneath, P.H.A. and R.R. Sokal. 1973. Numerical Taxonomy. WH Freeman, San Francisco.

Soleimani, V.D., Baum, B.R. and D.A. Johnson. 2003. Efficient validation of single nucleotide polymorphisms in plants by allelic specific PCR within an example from barley. Plant Mol. Bio. Rep. 21: 281-288.

Twumasi-Afriyie, S., Z. Habtamu, Y. Kassa, A. Bayisa, and T. Sewagegne. 2001. Development and Improvement

of Highland Maize in Ethiopia, pp 31-38. Enhancing the Contribution of Maize to Feed Security in Ethiopia. Proceedings of the Second National maize Workshop of Ethiopia. 12-16 November 2001, Addis Ababa, Ethiopia.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T.V. Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kulper, and M. Zabeau. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4007- 4414.

Warburton, M.L., X. Xainchun, J. Crossa, J. Franco, A.F. Melchinger, M. Frisch, M. Bohn, and D. Hoisington. 2002. Genetic characterization of CIMMYT Inbred maize inbred lines and open pollinated populations using large scale fingerprinting methods. Crop Sci. 42: 1832-1840.

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