CHAPTER 3

THE PREDICTION OF BEST LINE COMBINERS AND HETEROSIS IN TANZANIAN MAIZE BREEDING LINES THROUGH THE USE OF AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLPs)

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ABSTRACT

Amplified fragment length polymorphism DNA markers have been used to assist plant breeders in the choice of maize parents for commercial hybrid production. However, maize yield in Tanzania is significantly reduced by gray leaf spot (GLS) disease, which is now regarded as the most yield limiting disease of maize worldwide. Thus combining GLS resistance genes and high yielding traits in hybrids is an important breeding strategy. The main aim of this study was to assess the genetic diversity of Tanzanian germplasm and to predict the potential of these inbreds in producing high yielding GLS resistant hybrids. Furthermore, the potential of such data to predict the best line combiners was investigated. This research used AFLP-DNA fingerprinting data from 21 moderately and highly GLS resistant maize inbreds. Results revealed that the genetic distance (GD) of some intergroup crosses were as high as 0.5. Theoretically, these intergroup hybrids with high GD could potentially produce high yielding GLS resistant hybrids. However, such hybrids would require field-testing in order to confirm these observations. Finally the results revealed high f values consistent with other reports in maize. In summary these results also corroborates the usefulness of AFLP in genetic diversity studies of germplasm, prediction of best line combiners and high heterosis level for commercial maize hybrid production.

Keywords: Coancentry coefficient (f), genetic distance, inbreds, interpopulation, intrapopulation,

Introduction

Development of inbred lines, followed by their assignment into appropriate heterotic groups and planning the desired crosses for superior hybrid production requires adequate knowledge of genetic diversity (Hallauer *et al.,* 1988). This applies particularly to maize hybrid breeding where recognition and exploitation of heterotic patterns between genetically dissimilar sources of germplasm are important for success and also when it comes to proprietary issues and plant variety protection. Genetic diversity in cereal collections is critical in finding new alleles that will improve yields to fight world hunger (Warburton *et al.,* 2002). Even unadapted parents with poor phenotypes can contribute favorable alleles to their progenies when these adapted genotypes are placed in favorable backgrounds. Therefore screening based on phenotypes may miss much of the favorable variation, and thus new allelic variation may be identified by means of markers and can be complimented with phenotypes (Tanksley & McCouch, 1997).

Various DNA based fingerprinting techniques are available for genetic variability studies (Liu & Cordes, 2004; Botha *et al.,* 2004; Kosman & Leonard, 2005). These provide powerful tools in the identification of genetically similar/dissimilar germplasm. In maize, RFLP have been used quite extensively for this purpose (Melchinger, 1993). However, RFLP assays are labour intensive and time consuming and therefore are increasingly substituted by other techniques based on the polymerase chain reaction such as AFLP and simple sequence repeats (SSR). AFLP are genomic fragments detected after selective amplification (Vos *et al.,* 1995). The major advantages of AFLP markers compared with markers like RFLP and SSR are the generation of multiple marker bands in a single assay without prior knowledge of sequence and are highly reproducible (Myburg *et al.,* 2001; Botha *et al.,* 2004). The usefulness of AFLP markers for genetic diversity studies has been demonstrated in many crops (Mackill *et al.,* 1996; Powell *et al.,* 1996; Pejic *et al.,* 1998).

Many maize breeding programs in Africa, for example, in Tanzania, has not yet been studied at a molecular level their breeding germplasm in order to know the extent of genetic diversity which are present in their working gene pools. Such information are important for biotic/abiotic resistance breeding and for the production of varieties/hybrids which are higher yielders and resistant to different stresses. Furthermore, such information could also help to show the composition of genetic variation in these materials as a strategy to identify sources of biotic and abiotic stress resistance. Finally, studies of this nature could help to suggest appropriate measures that should be taken to sustain the existing genetic variation and against depletion of the present gene pools. In this study, the genetic distances of inbreds using AFLPs were determined and thereafter intend to use this information to assess the level of genetic diversity of the Tanzanian gene pools. Also, to test the usefulness of AFLP for determining best line combiners for increased hybrid heterosis. Lastly to predict the potential of these inbreds to produce high yielding GLS resistant hybrids for commercial use.

Materials and methods

Plant material

Twenty-one inbred lines were selected from the maize breeding program in the Southern highlands (1500 and 2500 metres above sea level) of Tanzania which is located in the Mbeya region. This region produces more than 50% of the country's maize annual output (Lyimo per. comm.). These inbreds were analysed using AFLP analysis (Table 1). The selection of the lines was based on their previous performance in a screening test for GLS resistance. Three seeds of each inbred line were sown in a plastic pot containing approximately 1.5 kg mixture of four parts sterilized clay soil and one part sand soil. Approximately 120 grams of a compound fertilizer of N: P: K (2:3:2) was applied to each pot. This pot trial was conducted at the University of Pretoria Experimental farm during the 2000/2001-rain season. At the three to four leaf stage (when plants were about two weeks old), the youngest fresh leaves of each line were harvested and immediately put on ice for DNA extraction.

DNA extraction

Genomic DNA was extracted from leaves of each of the lines as described by Doyle & Doyle (1987). Total genomic DNA was extracted from approximately 100 mg of leaf tissue using 5% (w/v) Cetyltrimethyl ammonium bromide (CTAB) [0.1M Tris-HCL (pH 8.0), 1.4 MNaCl, 20 mM EDTA (pH 8.0), 1% (w/v) soluble Polyvinylpyrrolidone (PVP) and 0.2 % (v/v) 2-Mercaptoethanol). Genomic DNA of each inbred was precipitated by using either ice-cold isopropanol or 95 % ethyl alcohol. The DNA pellets were dissolved in either 100 µl double distilled water or in low TE buffer [10 mM Tris-HCL (pH 8.0), 0.1 mM EDTA] and then stored at –20 ^{0}C. Quantification of the genomic DNA was done using a spectrophotometer.

Table 1. Origin, pedigree and heterotic groups of maize lines used in this study.

N0	Name of a line	Kernel type	GLS Rating	Source of germplasm	Pedigree	Established heterotic group
1	K53015213	Flint	3.25	K205xK230	K530 $S_5$152-1-3	A
2	K5301482	Flint	3.00	K205xK230	K530 $S_5$1482 (97)-1	A
3	K53014821	Flint	2.25	K205xK230	K530 $S_5$14821(98)-1	A
4	P629521	Flint	2.75	Population 62	P62 s_7-95-2-1	A
5	P621111	Flint	2.50	Population 62	P62 S_7 11-1 –1	A/B
6	P627733	Flint	2.00	Population 62	P62 $s_6$77-3Pap3	A
7	P62L50	Flint	2.25	Population 62	P62 $s_6$50	A
8	P62145	Flint	1.50	Population 62	P62$s_6$145-95	A/B
9	CML37	Dent	2.25	Population 32	Pop32c4Hc128-1-1-B-H5	A
10	P621621	Flint	1.75	Population 62	P62$S_6$6-2-1 Pap2	A
11	P628495	Flint	2.50	Population 62	P62 $s_6$84-95Blk	A/B
12	P62103	Flint	8.00	Population 62	P62 103s_6-93	A
13	CML11	Dent	3.00	Population 21	Pop21c5hc219-3-2-2-3#7-1B-4-10b	A
14	E50932815	Dent	2.25	E250xE393	E5093 s_6Pap28-1-5	B
15	P621321	Flint	1.75	Population 62	P62 $s_7$13-2-1	A
16	K375891	Dent	2.50	K337xK358	K3758 $S_7$9-1-1-1	A
17	P62 101	Flint	3.50	Population 62	P62 101-95	A
18	P62148	Flint	2.50	Population 62	P62 $s_6$148-95	B
19	E5093642	Dent	3.50	E250xE393	E5093 $s_7$64	B
20	K37581011	Dent	3.25	K337xK358	K3758 $S_7$10-1-1	A
21	K3758L36	Dent	2.00	K337xK358 L393	K3758s_6L36	B

AFLP analysis

AFLP analysis was performed according to the protocol of Vos *et al*. (1995). Genomic DNA of the maize inbreds (approximately 100mg) was digested with restriction enzymes *EcoR*1 and *Mse*1. In a second step the following adaptor sequences were ligated to the restricted DNA fragments.

*EcoR*1: 5'-CTCGTAGACTGCGTACC
CATCTGACGCATGGTTAA-5'

*Mse*1: 5'-GACGATGAGTCCTGAG
TACTCAGGACTCAT-5'

The primers used for pre-amplification and amplification were similar to those described by Vos *et al.* (1995) with *EcoR*1/*Mse*1 extensions ACA/CTG, ACA/CAG, ACA/CCG, ACA/CCC and ACA/CGC (Table 2). The five best primer combinations out of 10 screened were chosen for the final selective amplification. *EcoR*1 primers were 5' labelled with infrared dye (1 µM IRDye700 or IRDye 800, LI-COR, Lincoln, NE, USA). The PCR cycle profile for selective amplification was as follows: one cycle of 94 ^{0}C for 10s, followed by 13 cycles of 65 ^{0}C for 30s, with 0.7 ^{0}C decrease/cycle. Then there was 23 cycles of 94 ^{0}C for 10s, 56 ^{0}C for 30s and 72 ^{0}C for 1 min, with 1s decrease /cycle. Finally, one cycle of primer elongation at 72 ^{0}C for 1 min.

Table 2. The five *EcoR*1/*Mse*1 primer pair combinations used in this study. *EcoR*1 was employed in combinations with the five *Mse*1 primers.

Name of primer	Primer sequence						
ACA-700	5' GAC	TGC	GTA	CCA	ATT	CAC	A-3'
*Mse*1-1	5'GAT	GAG	TCC	TGA	GTA	ACC	C-3'
*Mse*1-2	5'GAT	GAG	TCC	TGA	GTA	ACT	C-3'
*Mse*1-3	5'GAT	GAG	TCC	TGA	GTA	ACA	C-3'
*Mse*1-4	5'GAT	GAG	TCC	TGA	GTA	ACC	C-3'
*Mse*1-5	5'GAT	GAG	TCC	TGA	GTA	ACG	C-3'

Electrophoresis and image analysis

AFLP fragments were resolved in 8% LongRanger^{TM} polyacrylamide gels using the LI-COR IR2 automated DNA analyser (LI-COR, Lincoln, NE, USA). The gel images were scored in a binary matrix that recorded the presence of bands as a plus (+) and absence of bands as minus (-). Semi-automated scoring was performed with the SAGA^{MX}software (Version 3.23, LI-COR). Scores were also manually edited to make corrections to the automated score where necessary.

Data analysis

Polymorphic bands were scored as plus (+) and minus (-) and converted to 1 or 0 when compiled into a data matrix. The data matrix was used to perform cluster analysis on the basis of the average linkage method, known as the Unweighted Pair Group Method (UPGMA) using PAUP software (Swofford, 1993), while the "goodness of fit" of the clustering to the data matrix was determined by calculating the cophenetic correlation coefficient between dissimilarity and the cophenetic matrix derived from the dendrogram (Sneath & Sokal, 1973). Polymorphism information content (PIC) provides information on the informativeness of a marker or an estimate of the discriminatory power of the locus or loci, by taking into account, not only the number of alleles that are expressed, but also the relative frequencies of those alleles. PIC values in this study were calculated by algorithm: PIC or (the diversity index of Nei, 1973) =1- $\sum_{i=1}^{n} fi^2$ where f is the frequency of ith allele averaged across loci. The marker index (MI) was calculated for the AFLP markers by applying the formulae given by Powell *et al.* (1996) and Smith *et al.* (1997). MI = % polymorphism x PIC and % polymorphism = number of polymorphic bands/ total number of bands in that assay unit.

Calculation of coefficient of coancentry (f)

The estimates of coefficient of coancentry (f) were taken from Messmer *et al.* (1992), wherein f was calculated as described by Falconer & Mackay (1996). For all pair of lines without known parentage, f was set to zero. Two lines were designated as unrelated if their coancentry was smaller than 0.1. Accordingly, for two inbreds related by pedigree with coancentry f and unknown marker genotypes of their ancestors an estimates of their GDs can be obtained as: $\hat{GD} = \overline{GD}$ (1- f), and if the GD of two individuals is known their coancentry can be estimated by: $\hat{f}$ =1-GD/$\overline{GD}$ where $\overline{GD}$ refers to the mean genetic distance of unrelated homozygous lines from the respective germplasm group with regard to the investigated set of marker loci and GD is the genetic distance between the two individuals.

Estimation of genetic distances

Estimates of GD among the 21 line combinations within each set were computed for AFLP marker system using the formula given by Nei & Li (1979). $GD_{ij}= (N_i+N_j-2N_{ij})/(N_i+N_j)$. Here GD_{ij} is the genetic distance between two inbred lines i and j. N_{ij} is the number of common bands in line i and j, and N_i and N_j are the total number of bands in line i and j respectively, with regard to all primer pair combinations of AFLP. Thus, GD reflects the proportion of bands in common between the two inbred lines and it may range from 0 (identical profile for two inbreds) to 1 (no common bands).

The Dendrogram

UPGMA was used to produce the dendrogram on the basis of AFLP markers and 21 inbreds (Figure 1), rather than Ward since the main aim of this research was to study the over all pattern of genetic diversity and not to maximize distance between the morphological traits as in case of populations. Warburton *et al.* (2002) used UPGMA to analyze CIMMYT lines for the same reason.

Results

Degree of polymorphism

The level of polymorphism was measured by the number of polymorphic bands, percent polymorphism, polymorphic index content, and marker index as shown in Table 2. The average marker index and the average PIC values for all five primer combinations were 21.4 and 0.26, respectively. Results showed that the largest marker index (24.9) was revealed by the primer pair combination *EcoR*1-ACA+*Mse*1-CCC while the assay unit *EcoR*1-ACA+*Mse*1-CGC showed the lowest index (16.5). The marker indices for the remaining assay units were in between. The total number of fragments (monomorphic and polymorphic bands) per primer pair combination varied from a minimum of 38 (primer pair combination *EcoR*1-ACA+*Mse*1-CGC) to a maximum of 67 fragments (primer pair combination *EcoR*1-ACA+*Mse*1-CCC). The total number of all AFLP markers in this study was 250 fragments, of which 208 fragments were polymorphic and the fragment sizes ranged from 50 base pairs to 456 base pairs (primer combinations not shown). Furthermore, results showed that the highest PIC (0.30) value was showed by the primer pair combination *EcoR*1-ACA+*Mse*1-CAG and primer pair combination *EcoR*1-ACA+*Mse*1-CGC revealed the lowest PIC (0.22) value. The PIC values for the remaining primer pair combinations were in between.

Table 3. Degree of polymorphism and information content for five primer combinations applied to 21 Tanzanian lines.

*EcoR*1	*Mse*1	Total fragments	Polymorphic bands	%Polymorphism	PIC	MI
1.ACA	CCC	67	58	86	0.29	24.9
2.ACA	CTG	40	34	84	0.26	21.8
3.ACA	CAG	50	40	80	0.30	23.2
4.ACA	CCG	55	47	85	0.24	20.4
5.ACA	CGC	38	29	75	0.22	16.5
Total		250	208	-	-	-
Average		50	41.6	82	0.26	21.4

MI=Marker index = % Polymorphism x PIC.

% Polymorphism= (Polymorphic bands x 100)/ Total fragments.

Genetic distance between inbred combinations of related and unrelated lines

Comparison of GD estimates between inbreds from the same and different heterotic groups were restricted to pairs of unrelated ($f = 0$) lines to minimize confounding effects due to relatedness (Table 4)**.** Genetic distance estimates for the 210 line combinations of 21 inbreds ranged from a minimum of 0.14 (intrapopulation) to a maximum of 0.45 (interpopulation) with a standard deviation for individual estimates varying from 0.02 to 0.06, respectively. Genetic distance estimates for unrelated line combinations within population 62 ranged from 0.14 to 0.33; of type K530 x K530 was from 0.15 to 0.18 and K3758 x K3758 was between 0.17 and 0.20 with a mean of 0.23, 0.16 and 0.19, respectively. Estimates of interpopulation line combinations varied from a minimum of 0.16 to a maximum of 0.45. Population 62 varied in their mean GD to K530 lines from 0.16 to 0.23 and to K3758 from 0.19 to 0.23 as well. Generally, (Table 4) the mean genetic distance of interpopulation (crosses between heterotic groups) (0.32) was much higher than the mean GD of crosses in intrapopulation groups (0.19) (crosses within heterotic groups).

Table 4. Summary of mean, maximum and genetic diversity of individual line combinations calculated from AFLP data using five primer combinations for various groups of related and unrelated pairs of 21 inbred lines.

Type of population	Crosses	#	Mean	Minimum	Maximum	SD	SE
A. Intrapopulations	1. Population 62 x Population 62	44	0.23	0.14	0.33	0.05	0.008
	2. K530 x K530	3	0.16	0.15	0.18	0.02	0.01
	3. K3758 x K3758	3	0.19	0.17	0.20	0.02	0.01
Mean (Intrapopulation)			0.19	0.15	0.23		
B. Interpopulations	1. Population 62 x K530	30	0.23	0.17	0.30	0.04	0.007
	2. K530 x K3758	9	0.24	0.16	0.27	0.04	0.01
	3. Population 62 x CML lines	20	0.31	0.25	0.36	0.03	0.006
	4. K530 x CML lines	6	0.25	0.16	0.32	0.06	0.02
	5. Population 62 x K3758	33	0.28	0.16	0.40	0.06	0.01
	6. Population 62 x Ecuador	22	0.32	0.21	0.38	0.05	0.01
	7. K530 x Ecuador	6	0.35	0.31	0.38	0.02	0.03
	8. K3758 crosses	12	0.34	0.25	0.42	0.06	0.01
	9. CML crosses	6	0.39	0.31	0.43	0.06	0.02
	10. Ecuador crosses	6	0.45	0.45	0.45	-	-
Mean (Interpopulation)			0.32	0.24	0.37		
Total		200					

= refers to the total number of individual crosses in that particular cross

Correlation coefficients between GD and coancentry coefficient (f)

The coancentry coefficient (f) and GD estimates represent fundamentally different concepts for measuring genetic diversity of related individuals (Table 4). Results showed that the coefficient of coancentry (f) for the flint type inbreds was 0.14, while the dent type lines exhibited a f value of 0.20. Furthermore, correlation coefficients between GD and the f values revealed that the flint type inbreds had a correlation value of –0.96. While the dent type inbreds produced a correlation coefficient between GD and coefficient of coancentry of (-0.94) respectively.

Table 5. Coefficient of coancentry and correlation coefficients (r) between genetic distances and coancentry coefficients (f) of maize lines.

Maize germplasm	Number	f†	r(GD, f)	Marker used
Pop 62 (Flint)	5PC	0.14	-0.96	AFLP
K3758 (Dent)	5PC	0.20	-0.94	AFLP

† = Mean of (f) values for all pairs considered.

PC= Number of primer pair combinations used

UPGMA dendrogram

The dendrogram or the genetic similarity/dissimilarity tree produced in this study is presented in Figure 1. In this dendrogram, there are three main distinct groups of inbred similarities. There is group 1. The lines in this group are: K53015213, K5301482 and K53014821**.** Group II, which is the largest group was comprised of lines developed from population 62. These lines were: P629521, P627733, P62L50, P628495, P62103, P62101, P62148, P621111, P621621, P621321 and lastly P62145. There were four subgroups of lines within group II. These subgroups were: Subgroup 1 (lines P629521, P627733, P62L50 and P628495). Subgroup 2. (lines P62103, P62101 and P62148). Subgroup 3 (lines P621111, P621621 and P621321). Then lastly, P62145 was an outlier in this main group. The third main group was comprised of lines which originated from a recombinant line K337 x K358. These lines were: K3758911, K37586911 and K37581011. Finally, the dendrogram revealed that the CIMMYT lines (CML37 and CML11) and the Ecuador lines (E50932815 and E5093642) were also outliers.

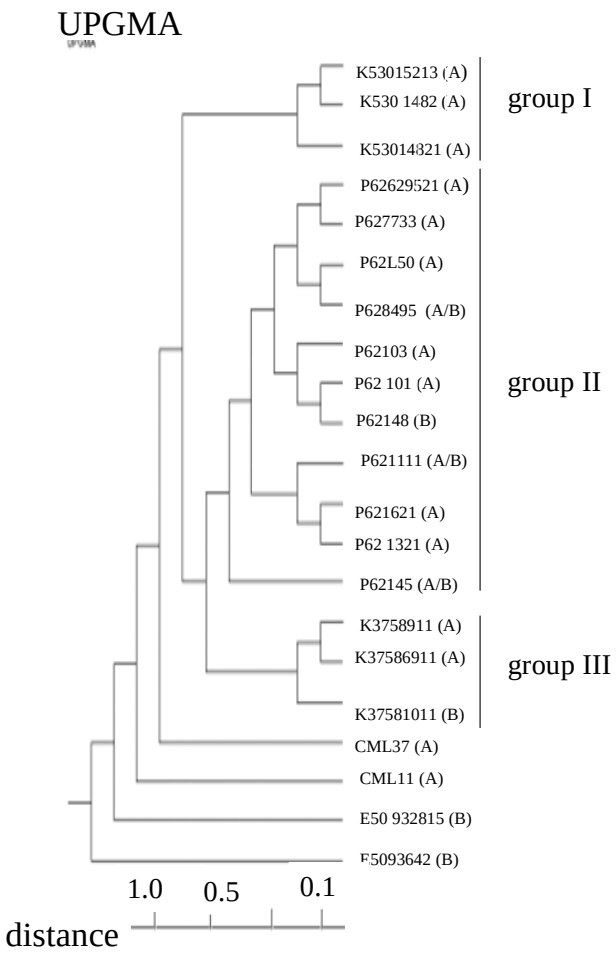

Figure1.The UPGMA dendrogram showing the grouping of twenty-one inbreds used in this study.

Discussion

In general, each of the five AFLP primer combinations used in this study discriminated effectively between most of the unrelated lines. The average marker index in this material was 21.4 (Table 3) which was slightly higher than that reported by Lübberstedt *et al.* (2000) in European maize. Their marker index was 16.4. The larger marker index in this study was attributable to the high average percent of polymorphic bands per lane, while the PIC values were about equal or slightly lower than those of Lübberstedt *et al*. (2000).

Similar results were reported for US maize germplasm (Pejic *et al.*, 1998), soybean (Powell *et al.*, 1996) and wheat (Bohn *et al.*, 1999). The higher marker index and PIC value of the AFLP markers in comparison with other marker systems like SSR, RFLPs, RAPDs, together with high reproducibility proves that AFLP markers are a valuable tool for identification of maize inbreds, plant variety protection and registration as well as patenting germplasm (Melchinger *et al.*, 1998a, b; Lübberstedt *et al.*, 2000). AFLP have added advantages in terms of reliability, reproducibility, discrimination, standardization and cost effectiveness. Furthermore, the results showed that primer pair *EcoR*1-ACA+*Mse*1-CAG was the most discriminative or informative because it revealed the highest PIC value (0.30) and primer pair *EcoR*1-ACA+*Mse*1-CGC was the least discriminative because it had the lowest PIC value (0.22).

One of the aims of this study was to test and evaluate inbreds as potential parents for developing commercial hybrids. It was demonstrated in the study that the GDs of genotypes from different germplasm groups (intergroup crosses) have on average significantly greater GDs than combinations of genotypes from the same germplasm group (intragroup crosses) (Table 4). This observation is consistent with the genetic theory, and those of Melchinger *et al.* (1998a, b) and Pejic *et al.* (1998) which states that intergroup crosses exhibit higher genetic distances between lines and lower genetic distances between lines in the intrapopulation crosses.

Breeders have used the coancentry [devised by Malecot's (1948)] as an indirect way of measuring genetic similarities between related individuals. It was observed that r (GD, f) values (Table 5) in both flint and dent kernel lines were much higher than in maize (Messmer *et al.*, 1993; Smith *et al.*, 1997) and other reported crops (Graner *et al.*, 1994; Schut *et al.*, 1997). The negative sign of the r (GD, f) means that in this case as one trait increases, the second tends to decrease. The main reasons for the higher r (GD, f) in this study could be due to fundamental differences between the two marker systems used. The AFLP marker system used in this study scores bands on a biallellic basis, while Messmer *et al*. (1993) and Smith *et al.* (1997) used RFLP, which usually scores multiple bands (alleles) per locus (Lübberstedt *et al.*, 2000). Secondly, the AFLPs are capable of

assaying more genetic loci than RFLP or SSR (Myburg *et al.*, 2001; Botha *et al.*, 2004; Liu & Cordes, 2004). Thirdly, the higher r (GD, f) could be due to the fact that AFLPs are able to amplify potentially more conserved organellar DNA sequences than the RFLP (Vuylsteke *et al*., 1999). Melchinger (1993) reported that high r (GD, f) estimates in maize suggest that if the pedigree data are correct, these correlations (GD, f) do provide reliable descriptors of genetic similarity in maize.

The dendrogram (Figure 1) revealed that the three recombinant inbreds, developed from K530 germplasm, clustered together and showed some level of genetic similarity with group II, which was comprised of inbreds originating from population 62. This implies that there are some genetic similarities between the recombinant inbreds derived from the K530 germplasm and those developed from population 62. This could be due to gene introgressions between the two sources of germplasm or between the inbreds *per se* or both. The intermingling of genes between the two sources could be via deliberate breeding or through contamination or both. These observations are vital to plant breeders in the sense that they should know the background history and the genetic purity of sources of germplasm they are working with. This, in turn, will help the breeders to develop inbreds without linkage drag that happens due to use of contaminated germplasm.

In general, the AFLP method could clearly distinguish all the inbreds and clustered them into appropriate groups according to the established pedigrees. However, there were a few discrepancies, especially in the placement of individual inbreds according to the source of germplasm from which they have been extracted. For example the grouping of all recombinant inbreds that were developed from K337 x K358 germplasm clustered with the group containing inbreds extracted from population 62. It was also observed that there were a lot of subgroups of inbreds derived from population 62, implying that this population is genetically diverse. Furthermore, line P62145, although clustered in-group II, did not cluster with any of the subgroups. Similar discrepancies of clustering of melon inbreds by using SSR within one germplasm source have been reported between population of origin and heterotic grouping, a result consistent with high level of genetic

diversity within source populations of inbreds (Monforte *et al*., 2003). The CIMMYT lines, which have different sources of germplasm, were grouped as expected. The Ecuador lines, however, according to their pedigree were supposed to group together but fell into different groups. This could be due to contamination or small mutations occurring in one of these lines.

Conclusions

AFLP markers for genetic diversity studies in maize have been employed to investigate the relationship between GD and hybrid performance/heterosis for yield and MPH (Melchinger *et al.*, 1998a, b; Lübberstedt *et al.*, 2000). In this study, however, it was observed that the AFLP markers have another added advantage of producing the best results for measuring correlations between coancentry coefficient and genetic distance of maize inbreds. It had the highest f values as compared to similar studies reported by Smith *et al.* (1997) and Messmer *et al.* (1993) etc. Furthermore, this study revealed that each line was uniquely identified when the five AFLP primer combinations were used and the grouping of the lines was almost consistent with their established pedigree (Figure 1). This research also showed that inbreds derived from intrapopulations as well as recombinants can have lower genetic distances than inbreds derived from open pollinated varieties (Table 4) and might suffer from the deleterious effect of inbreeding depression and degeneration. Finally, it is important to note that inbreds which are similar can only be crossed among themselves if (i) they can show an increased level of heterosis of the target trait; (ii) these inbreds could complement one another for those genes which are missing in each other and these genes are able to improve the agronomical traits of the resulting hybrids; and (iii) are needed to produce large quantities of seed which single crosses are not able to produce.

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