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Molecular Genetic Characterization of Eighteen Maize Inbred Lines by RAPD-PCR Markers

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ABSTRACT

Nine random primers were used to identify and characterize 18 maize inbred lines by randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis. The results indicated distinct differences can be used for identification of maize inbred lines. A total of 106 amplified DNA fragments ranging in size from 1529 to 163 base pairs were present, whereas 83 fragments were polymorphic and 23 fragments were monomorphic. The primer OP-B11 gave the highest number of polymorphic fragments in the 18 inbred lines (14 fragments) with 93% polymorphism. Six primers OP-A05, OP-A06, OP-A09, OP-B08, OP-B09 and OP-C15 produced eight specific DNA fragments identified six inbred lines. The similarity values showed substantial differences among the maize inbred lines. The genetic similarity ranged from 36 to 91%, with an average of 63.5%. The dendrogram resulting from the unweighted pair group method with arithmetic average (UPGMA) cluster analysis showed that the 18 maize inbred lines could be divided into two main clusters. The primers used in this study succeeded in distinguishing most of the studied inbred lines of maize in unique banding patterns when each primer is used alone. The number of DNA fragments for each primer varied from 8 (OP-B09) to 17 (OP-A05). The primer OP-A05 was the best one in distinguishing the maize inbred lines because it identified 15 inbreds, but to identify all inbreds we have to use more than one primer such as a combination of the three primers OP-A05, OP-A06 and OP-B08.

Keywords: dendrogram, genetic diversity, maize, molecular markers

INTRODUCTION

Molecular markers are used in genetic improvement in different fields of agricultural research. The simplicity of random amplified polymorphic DNA (RAPD) makes it ideal for genetic mapping, plant and animal breeding programs, and DNA fingerprinting, with particular utility in the field of population genetics (Salem *et al*. 2007). Lun *et al*. (2000) identified 46 inbred lines (ILs) of maize widely used in China using RAPD molecular markers and their results showed that these ILs could be distinguished from each other just by the combination of amplification products of 6 primers (A6, C6, D2, F10, H19 and N19) without the need to select special molecular markers for each IL. Hua *et al*. (2000) examined the seeds of 12 elite maize ILs by means of RAPD analysis. Among the 250 Operon primers screened, 12 gave reproducible and polymorphic DNA amplification patterns. Eleven bands amplified by primer OPN-11 were used in the development of RAPD DNA fingerprinting of the 12 ILs. Each IL had a unique fingerprint allowing it to be easily distinguished from other lines. The RAPD DNA fingerprinting analysis developed by this research can be used in practical seed identification of the 12 elite maize ILs, for both true and false as well as purity determination. Young *et al*. (2004) carried out a study to investigate the major agronomic traits of newly developed sweet corn ILs and to analyze their genetic similarity using RAPD. A total of 100 RAPDs primers were used to detect the polymorphisms in 25 ILs. Of these primers, 54 primers showed polymorphisms and total number of polymorphic bands was 256. Six primers, OPA09, OPA17, OPB04, OPB07, OPB11 and OPC04, produced specific DNA fragments. A dendrogram was constructed using UPGMA cluster analysis from the RAPD banding profiles, which the 25 ILs were separated into four groups.

Bruel *et al.* (2007) used RAPD markers to analyze genetic diversity between 16 corn lines. Twenty-two primers were used resulting in the amplification of 265 fragments, of which 237 (84.44%) were polymorphic. Using the UPGMA method, the genetic associations obtained showed 5 distinct heterotic groups. The high rate of polymorphism between lines revealed by RAPD markers indicated that the method is efficient to analyze genetic diversity in corn lines and that the genetic divergence can be used to establish consistent heterotic groups between corn lines. Okumus (2007) carried out a comparative characterization of 17 Turkish flint maize landraces (*Zea mays* L.) using RAPD markers. Fourteen primers giving reliable and consistent polymorphic bands amplified 125 fragments with an average of 8.90 fragments per primer. Genetic variation in 17 maize landraces was characterized based on dissimilarity matrix by UPGMA dendrogram which has no association into distinct grouping with respect to locations and many small clusters formed. The similarity was in range from 0.05 to 0.88. The Turkish flint accessions had a high variability crossing from the different maize genetic resources.

Thakur *et al.* (2008) employed PCR-based RAPD markers to assess the genetic diversity among 10 maize cultivars. Most of the primers showed a single polymorphic band, and 92.92% of the products were polymorphic. Seventy-four fragments were obtained, and an average of 5.3 bands per primer and mean of 5.1 polymorphic bands were recorded. Based on polymorphism, 5 RAPD primers (OPD-05, OPC-08, OPP-16, OPE-03 and OPF-17) were found to be highly discriminative. Genetic similarity based on Jaccard's similarity coefficient ranged from 0.214 to 0.725, indicating narrow genetic variability among the genotypes based on RAPD markers. The 10 cultivars of maize formed 2 major clusters in the dendrogram. Souza *et al.* (2008) used the RAPD markers to compare the genetic

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Table 1 The 18 maize ILs used in the study and their origins.

Maize ILs	Origin
K1	Single Cross 10 (S.C.10)
K5	Nab El_Gamal (local open pollinated cultivar)
K7	Giza 2-synthetic variety
K8	Giza 2-synthetic variety
K10	Single Cross 10 (S.C.10)
K11	Girga Balady (local open pollinated cultivar)
K51	Single Cross 10 (S.C.10)
K61	Taba (double cross variety)
K62	Single Cross 10 (S.C.10)
Rg18	G.S. (PI221866 X 303) X (216 X mo2rf)
Rg25	G.S. (Syn.Laposta X Ci-64) X SC.14
Rg33	G.S. (PI221866 X 307) X S.C.14
Rg59	G.S. (Syn.Laposta X 307) X S.C.14
G227b	TuxPan
G244C	Laguna
G307A	S. C. U. 1201
G342	LocBrod SC12100 USA
L162B-2	Locally developed

Table 2 List of nine random primers and their nucleotide sequences.

Primer	Sequence (5'-3')
OPA-05	AGGGGTCTTG
OPA-06	GGTCCCTGAC
OPA-09	GGGTAACGCC
OPB-05	TGCGCCCTTC
OPB-08	GTCCACACGG
OPB-09	TGGGGGACTC
OPB-11	GTAGACCCGT
OPB-14	TCCGCTCTGG
OPC-15	GACGGATCAG

diversity among 16 maize ILs. Twenty-two primers were used in the RAPD reactions, resulting in the amplification of 265 fragments. The similarity based on Dice coefficient for the RAPD ranged from 53 to 84%. The dendrogram obtained showed five groups indicating that the RAPD was effective to validate pedigree data. Leal *et al.* (2010) quantified genetic diversity among 10 inbred popcorn lines using both RAPD and SSR markers, and evaluated how well these two types of markers discriminated the popcorn genotypes. They found that both techniques are efficient for evaluating genetic diversity in the genotypes of popcorn that evaluated, though RAPDs yielded more polymorphisms.

The objective of this study was to estimate the genetic diversity of 18 Egyptian maize ILs using RAPD-PCR analysis.

MATERIALS AND METHODS

Materials

Eighteen Egyptian maize ILs (*Zea mays* L.) with different genetic background, as shown in **Table 1**, were used in this study. ILs 1 to 9 were developed by the plant breeding group, Genetics and Cytology Dept., National Research Centre, Cairo, Egypt. ILs 10 to 18 were kindly furnished by the Maize Research Section, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt.

Methods

Ten grains from the 18 maize ILs were germinated on wet filter papers for two weeks and the genomic DNA was extracted from 0.5 g of young and fresh leaves following Dellaporta method (Dellaporta *et al.* 1983). RAPD markers are produced by PCR using the genomic DNA and arbitrary primers (Devos and Gale 1992). PCR reactions were conducted using nine Operon primers (Metabion International AG, Martinsried, Germany) as shown in **Table 2**, to characterize the 18 Egyptian maize ILs. PCR technique was performed in 25 µl volume containing the following: 10 µl master mix, 10 µl buffer (10 X), 1 µl primer (100 pmol), 1 µl DNA template (50 ng) and 3 µl H_2O sterile. The amplification was carried out in a thermocycler programmed as follows: 1 cycle of 94°C/2 min, 30 cycles of (94°C/1 min, 37°C/2 min, 72°C/2 min), 1 cycle of 72°C/7 min.

Agarose gel was used for separating the PCR products of amplified DNA fragments by electrophoresis. The agarose gel was prepared by dissolving 1 g agarose in 100 ml buffer including 40 mM Tris acetate and 2 mM $Na_2EDTA.2H_2O$. Each gel was run 2-3 times with 2 or 3 independent samples for each primer; the best one in the RAPD banding profile was chosen for analysis. The gel was photographed and scanned using a Gel-Documentation system. Data were analyzed using Bio-Rad Model 620 Software Programs, USA. Genetic similarity was estimated using Nei-Li's similarity index (Nei and Li 1979). A dendrogram was constructed on the basis of the similarity matrix data by the unweighted pair group method with arithmetic average (UPGMA) cluster analysis using the software MEGA program.

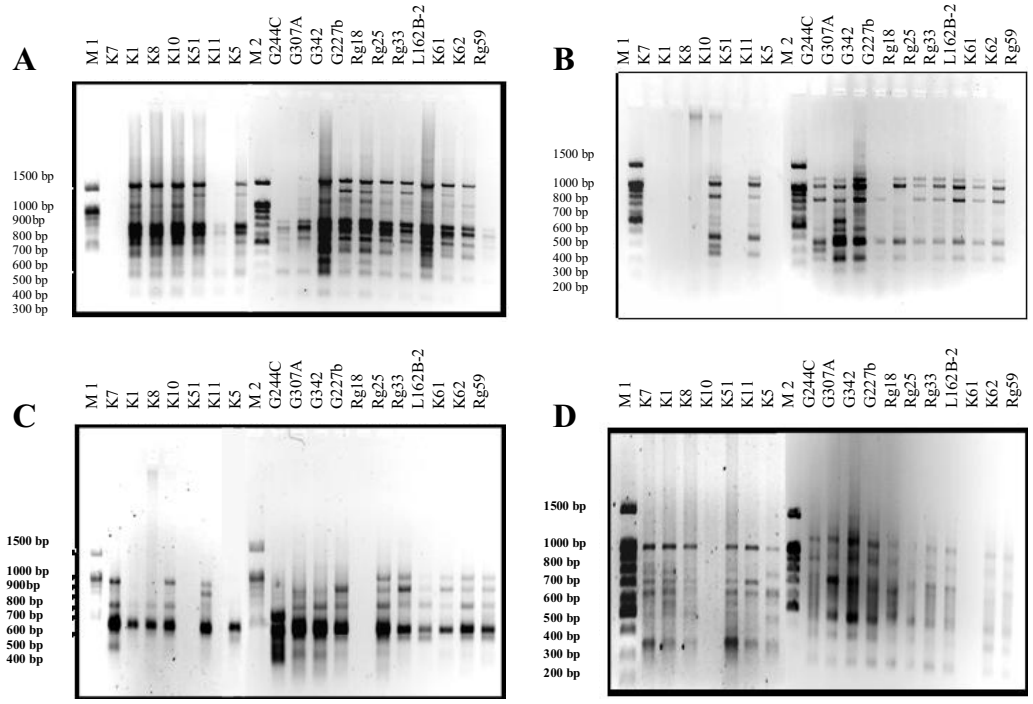

Fig. 1 Electrophoretic profile of PCR products using OP-A05 (**A**), OP-A06 (**B**), OP-A09 (**C**) and OP-B05 (**D**) random primers for 18 ILs of maize. M1 and M2: DNA markers.

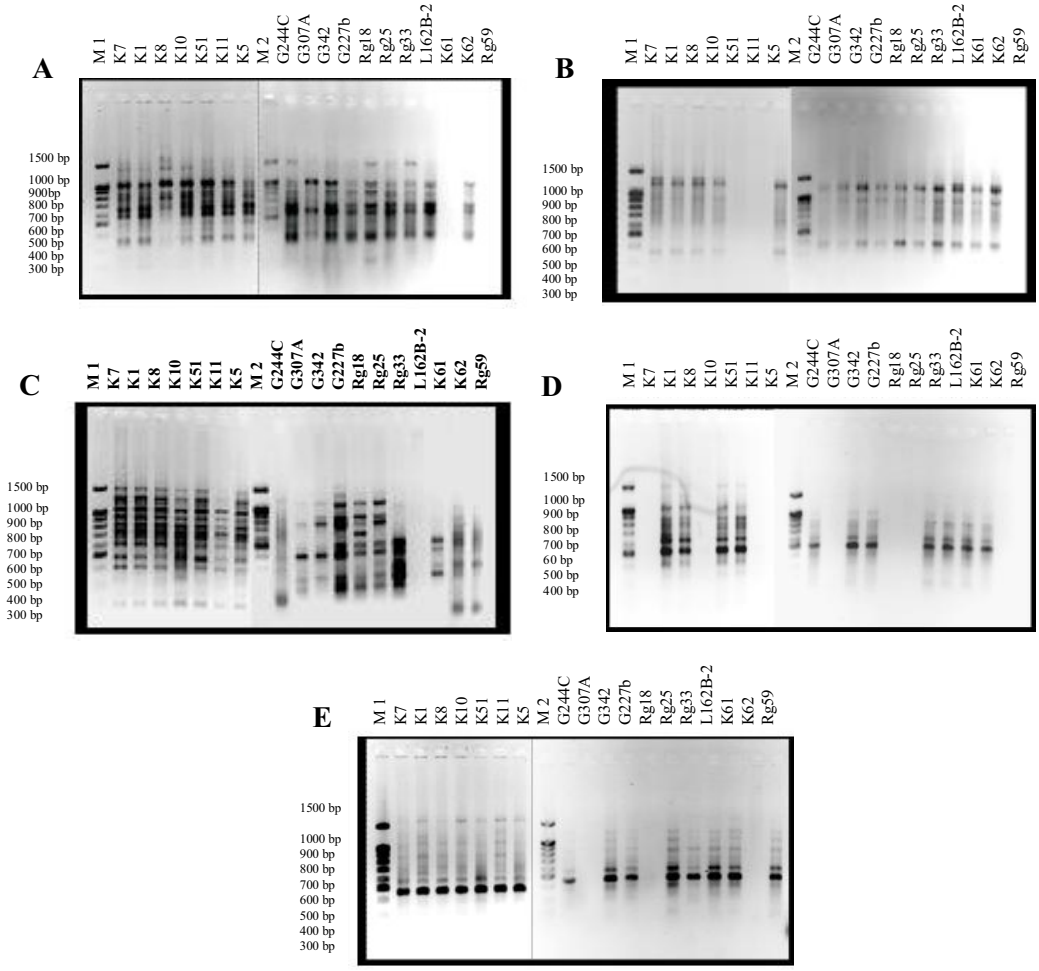

Fig. 2 Electrophoretic profile of PCR products using OP-B08 (**A**), OP-B09 (**B**), OP-B11 (**C**), OP-B14 (**D**) and OP-C15 (**E**) random primers for 18 ILs of maize. M1 and M2: DNA markers.

Table 3 Total bands produced from RAPD-PCR analyses for the 18 maize ILs using nine primers.

Maize ILs	Primers									*
	OP-A05	OP-A06	OP-A09	OP-B05	OP-B08	OP-B09	OP-B11	OP-B14	OP-C15	
K7	-	-	6	7	11	7	13	5	-	49
K1	13	-	2	7	11	5	13	8	9	68
K8	12	-	3	6	8	6	14	7	10	66
K10	14	10	4	-	11	5	15	8	-	67
K51	10	-	-	8	12	-	15	6	9	60
K11	5	7	5	6	10	-	12	8	9	62
K5	10	-	2	6	8	5	15	6	-	52
G244C	7	9	7	8	11	3	8	3	5	61
G307A	11	10	6	8	7	4	8	-	-	54
G342	15	12	5	9	10	6	7	8	6	78
G227b	15	4	6	9	8	4	15	8	6	75
Rg18	14	8	-	8	10	4	9	-	-	53
Rg25	13	5	6	5	8	6	12	8	-	63
Rg33	14	7	5	7	9	8	8	4	6	68
L162B-2	17	8	5	7	7	7	-	8	6	65
K61	14	6	6	-	-	6	8	9	6	55
K62	11	7	5	6	5	6	9	-	6	55
Rg59	6	-	6	6	-	-	4	7	-	29
Total bands	201	93	79	113	146	82	185	103	78	1080

* Refers to the presence of all amplified fragments in each line

RESULTS AND DISCUSSION

Identification of maize ILs

Nine random primers were used to identify and characterize the 18 Egyptian maize ILs. **Fig. 1** and **2** show the banding patterns produced from each primer for the 18 maize ILs. The highest number of PCR-amplified fragments was present in the inbred 'G342' (78 fragments), while the inbred 'Rg59' gave the lowest number (29 fragments), as shown in **Table 3**. The other ILs displayed different numbers of amplified fragments. On the other hand, the primer OP-A05 gave the highest number of amplified fragments (201 fragments), while the primer OP-C15 showed the lowest number of amplified fragments (78 fragments) in the studied ILs. RAPD specific markers with molecular weights of 392, 389, 245, 492 and 1393 bp were detected in the electrophoretic patterns of ILs 'L162B-2', 'G244C', 'Rg18', 'Rg33' and

Table 4 Polymorphisms revealed by nine primers used for identification of the 18 maize ILs.

Primers	Total bands	Polymorphic	Monomorphic	Polymorphism (%)	Specific bands
OP-A05	17	13	4	76	1
OP-A06	13	10	3	77	3
OP-A09	9	8	1	89	1
OP-B05	12	10	2	83	-
OP-B08	13	10	3	77	1
OP-B09	8	6	2	75	1
OP-B11	15	14	1	93	-
OP-B14	9	7	2	78	-
OP-C15	10	5	5	50	1
All primers	106	83	23	78.3	8

Table 5 Genetic similarity percentages of the 18 maize ILs based on RAPD banding patterns.

Maize ILs	K7	K1	K8	K10	K51	K11	K5	G244C	G307A	G342	G227b	Rg18	Rg25	Rg33	L162B-2	K61	K62	Rg59
K7	1																	
K1	72	1																
K8	71	91	1															
K10	64	76	71	1														
K51	62	84	81	65	1													
K11	64	78	73	72	76	1												
K5	75	77	76	76	77	64	1											
G244C	58	63	63	65	66	72	60	1										
G307A	52	54	55	68	51	53	62	72	1									
G342	57	71	68	73	64	70	65	74	74	1								
G227b	63	81	78	76	74	74	74	76	71	86	1							
Rg18	49	61	59	70	60	55	67	70	80	75	70	1						
Rg25	68	69	70	79	62	67	75	68	76	80	83	73	1					
Rg33	58	69	70	71	63	66	65	71	75	85	83	74	78	1				
L162B-2	49	66	64	68	53	66	56	66	66	85	79	66	76	86	1			
K61	44	61	64	72	50	61	56	54	58	78	75	55	75	77	76	1		
K62	53	63	66	67	57	67	61	68	73	75	75	70	73	79	76	71	1	
Rg59	43	45	48	43	42	52	46	50	43	44	53	36	57	43	48	47	49	1

'K8' using the primers OP-A05, OP-A09, OP-B08, OP-B09 and OP-C15, respectively. In addition, three specific RAPD markers with molecular weights of 1500, 1409 and 916 bp were detected in the electrophoretic pattern of 'G342' IL using the primer OP-A06. No RAPD specific markers were detected using the primers OP-B05, OP-B11 and OP-B14. Therefore, six primers out of the nine used in this study produced specific amplified fragments which can be used to identify the ILs 'K8', 'G244C', 'G342', 'Rg18', 'Rg33' and 'L162B-2'. Four common fragments were detected in electrophoretic patterns of 17 ILs using primer OP-A05, one common fragment was present in patterns of 16 ILs when primers OP-A09 and OP-B11 were used, two common bands in patterns of 16 ILs were found using primer OP-B05 and three common fragments in patterns of 16 ILs were observed using primer OP-B08.

The polymorphism revealed by the nine primers used for identification of the 18 maize ILs is shown in **Table 4**. The primer OP-B11 gave the highest number of polymorphic fragments in the 18 ILs (14 fragments) with 93% polymorphism, while the primer OP-C15 gave the lowest number of polymorphic fragments (5 fragments) with 50% polymorphism. The results indicated distinct differences for identification of maize ILs. A total of 106 amplified DNA fragments ranging in the size from 1529 to 163 base pairs were present, whereas 83 fragments were polymorphic and 23 fragments were monomorphic. Therefore, out of 106 amplified products, 21.7% were monomorphic and 78.3% were polymorphic.

The DNA primers used in RAPD-PCR analysis succeeded in distinguishing most of the studied maize ILs in unique banding patterns when used each primer alone. The primer OP-A05 identified 15 ILs, OP-A06 (8 ILs), OP-A09 (5 ILs), OP-B05 (6 ILs), OP-B08 (11 ILs), OP-B09 (6 ILs), OP-B11 (11 ILs) and OP-B14 (8 ILs). Therefore, the primer OP-A05 was the best one in distinguishing the maize ILs, but to identify all ILs we have to use more than one primer such as a combination of the three primers OP-A05, OP-A06 and OP-B08. The high rate of polymorphism between ILs revealed by RAPD markers indicated that the method is efficient to analyze genetic diversity in maize ILs.

These results are in agreement with those of Ajmone *et al.* (1993) who observed that RAPD method was originally developed for identification of clones (fingerprinting), and its use in genetic analysis of maize; Lun *et al*. (2000) who identified 46 ILs of maize widely used in China using RAPD molecular markers and they showed that these ILs could be distinguished from each other just by the combination of amplification products of 6 primers (A6, C6, D2, F10, H19 and N19) without the need to select special molecular markers for each inbred. Hua *et al*. (2000) examined seeds of 12 elite maize ILs by means of RAPD analysis. Among the 250 Operon primers screened, 12 gave reproducible, polymorphic DNA amplification patterns. Eleven bands amplified by primer OPN-11 were used in the development of RAPD-DNA fingerprinting of the 12 ILs. Each IL had a unique fingerprint allowing it to be easily distinguished from other ILs. Salem *et al*. (2007) reported that the RAPD technique is ideal for genetic mapping, plant and animal breeding programs, and DNA fingerprinting, with particular utility in the field of population genetics.

Genetic similarity of different maize ILs using RAPD markers

The genetic similarity index and dendrogram tree of the 18 maize ILs under study were performed using Nei-Li's similarity index on the basis of RAPD amplified fragments as presented in **Table 5** and **Fig. 3**. Similarity values showed clearly substantial differences among the maize ILs. The genetic similarity ranged from 36 to 91%, with an average of 63.5%. Some ILs showed high genetic similarity with others, such as 'K1' and 'K8' (91%), 'G342' and 'G227b' (86%), 'Rg33' and 'L162B-2' (86%). On the contrary, some ILs displayed low genetic similarity, such as 'Rg18' and 'Rg59' (36%), 'K51' and 'Rg59' (42%). The other ILs displayed different genetic similarities.

The dendrogram resulting from the UPGMA cluster

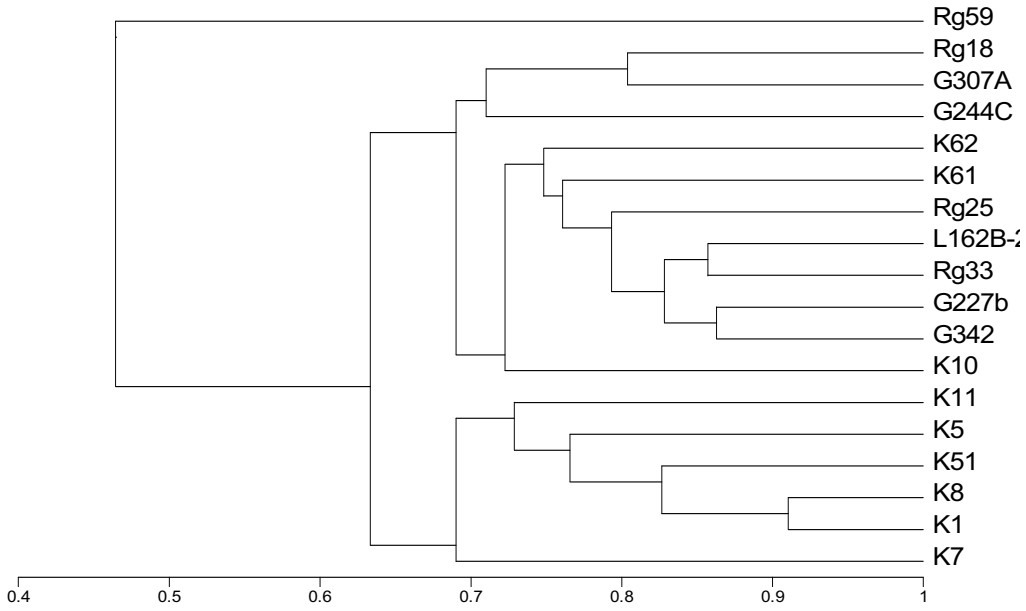

Fig. 3 dendrogram representing the genetic relationships among the 18 maize ILs using UPGMA cluster analysis of Nei-Li's similarity coefficient generated from RAPD markers.

analysis showed that the 18 maize ILs could be divided into two main clusters. The first cluster contained six ILs, while the second cluster contained 11 ILs. The first cluster contained ILs 'K1' and 'K8' with similarity 91% grouped with ILs 'K7' and 'K11' followed by ILs 'K51' and 'K5'.

The second cluster was divided into two sub-clusters; the first contained eight ILs, the two ILs 'G342' and 'G227b' with similarity 86% grouped with the two ILs 'Rg33' and 'L162B-2' with also similarity 86% followed by the ILs 'Rg25', 'K61' and 'K62' grouped with 'K10'. While the second sub-cluster included three ILs, the two ILs 'G307A' and 'Rg18' with similarity 80% grouped with 'G244C'. The IL 'Rg59' was located out of all ILs in the dendrogram.

These results agreed with those of Souza *et al.* (1993) who used RAPD markers to evaluate the genetic divergence of maize ILs, and Shieh and Thseng (2002) who showed that RAPD analysis was a useful tool in determining the extent of genetic diversity among Tainan-white maize ILs, and the ILs could be classified into distinct heterotic groups. Young *et al*. (2004) found, from a dendrogram was constructed using UPGMA cluster analysis from the RAPD banding profiles for sweet corn, that 25 ILs were separated into four groups.

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