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Full Length Research Paper

Assessment of genetic diversity in Sudanese maize (*Zea mays* L.) genotypes using random amplified polymorphic DNA (RAPD) markers

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The randomly amplified polymorphic DNA (RAPD) molecular markers were used to assess genetic diversity in 27 Sudanese maize genotypes. Ten primers were used, resulting in the amplification of 59 fragments, of which 53 (89.33) were polymorphic. The maximum number of fragment bands (10) were produced by the primer A-1 with 100% polymorphism, while the minimum numbers of fragments (3) were produced by the primer OPA-20. Using the unweighted pair group method with arithmetic averages (UPGMA) method, the genetic associations obtained showed three distinct heterotic groups. The high rate of polymorphism between genotypes revealed by RAPD markers indicated that the method is efficient to analyze genetic divergence and can be used to establish consistent heterotic groups between maize genotypes.

Key words: Randomly amplified polymorphic DNA (RAPD) markers, DNA polymorphism, maize genetic diversity.

INTRODUCTION

Maize (*Zea mays* L.) is the third most important cereal crop in the world after rice and wheat. It is cultivated in a wider range of environments than wheat and rice because of its greater adaptability (Koutsika-Sotiriou, 1999). In Sudan, maize is normally grown as a rain fed crop in Kordofan, Darfur and Southern states or in small-irrigated areas in Northern states (Ahmed and Elhag, 1999).

The increasing demand for maize for poultry feed or intermediary products for human nutrition have led to greater interest in this crop in Sudan. However, the relatively narrow gene pool and the heavy use of a small number of parents by competing breeding programs have led to a low genetic diversity among maize cultivars. Extensive use of closely related cultivars by producers could result in vulnerability to pests and disease (Duvick, 1984). Determination of the genetic diversity of any given crop species is a suitable precursor for improvement of the crop because selection of the desirable genotypes for a certain trait will not be effective unless considerable genetic variation is existing in the material under study (Khalafalla and Abdalla, 1997).

Different methodologies have been used to characterize genetic diversity in the maize germplasm, which are morphological characters (Goodman and Bird, 1977), pedigree analysis (Duvick, 1984), heterosis (Smith and Smith, 1989) and the detection of variation at the DNA level using markers.

The genetic diversity evaluation by the means of the molecular markers presents some advantages over other methods because, in addition to identifying the high polymorphism, they do not present interactions with the environment, and can be evaluated at any stage of development (Williams et al., 1990). Among the different types of molecular markers, randomly amplified polymorphic DNAs (RAPDs) are useful for the assessment of genetic diversity (Williams et al., 1990) because of their

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Table 1. List of maize genotypes studied and their types.

Key word	Genotype	Type
1	66y	In bred line
2	277	In bred line
3	3	In bred line
4	6	In bred line
5	160y	In bred line
6	2	In bred line
7	405	In bred line
8	Huediba -1	Hybrid
9	Huediba -2	Hybrid
10	6 × 3	Hybrid
11	277 × 6	Hybrid
12	66 × 405	Hybrid
13	277 × 66	Hybrid
14	3 × 405	Hybrid
15	66 × 160	Hybrid
16	160 × 277	Hybrid
17	2 × 160	Hybrid
18	405 × 160	Hybrid
19	405 × 277	Hybrid
20	66 × 3	Hybrid
21	2 × 277	Hybrid
22	66 × 6	Hybrid
23	160 × 6	Hybrid
24	6 × 2	Hybrid
25	160 ×3	Hybrid
26	66 × 277	Hybrid
27	3 × 2	Hybrid

simplicity, speed and relatively low-cost (Rafalski and Tingey, 1993) as compared to other types of molecular markers. RAPD can be used in studying genetic diversity, phylogeny, quantitative trait loci and varietals identification (Weising et al., 1995). In maize, this technique has been widely used in diversity studies because, in addition to its low cost, it allows polymorphism to be detected in a simple and fast manner (Liu et al., 1998; Wu, 2000). Therefore, the objective of this study was to analyze genetic diversity between 27 maize genotypes by means of RAPD markers, and to estimate the genetic distance among the inbred line and hybrids.

MATERIALS AND METHODS

Twenty seven (27) maize genotypes including twenty (20) hybrids and seven inbred lines obtained from Dr. Abdelwahab H. Abdalla, Faculty of Agriculture, University of Khartoum were used in this study (Table 1). Seeds of all genotypes were sown separately in pots and leaf samples pooled from all plants of each genotype were collected into labeled bags and used for genomic DNA Isolation.

DNA extraction

Genomic DNA was extracted from fresh leaf tissue of 27 individuals using modified CTAB method (Porebski et al., 1997). In this method, the fine powdered plant materials were immediately transferred into 13 ml Falcon tubes containing 6 ml of pre-warmed lysis solution. Tubes containing the samples were then incubated in a water path at 65°C with gentle shaking for 30 min and left to cool at room temperature for 5 min. Isoamyl and chloroform mixture (1:24) was added to each tube and the phases were mixed gently for 5 min at room temperature to make a homogenous mixture. The cell debris was removed by centrifugation at 5000 rpm for 15 min and the resulted clear aqueous phases (containing DNA) were transferred to new sterile tubes. Chloroform : isoamyl alcohol extraction was repeated twice. The nucleic acids in the aqueous phase were precipitated by adding equal volume of deep cooled

Table 2. Polymorphism detected by the use of 10 random primers on 27 (*Zea mays* L) genotypes.

Name of primer	Sequence of primer (5′- 3′)	Total number of bands	Number of polymorphic band	Percentage of polymorphic bands (%)
A-1	CAGGCCCTTC	10	10	100
B-7	GGTGACGCAG	5	4	80
C-2	GTGAGGCGTC	10	9	90
C-8	TGGACCGGTG	5	5	100
D-20	ACCCGGTCAC	5	4	80
OPA-17	GACCGCTTGT	5	5	100
OPA-20	GTTGCGATCC	3	3	100
UBC-101	GCGGCTGGAG	5	4	80
UBC-106	CGTCTGCCCG	6	5	83.3
UBC-155	CTGGCGGCTG	5	4	80
Total		59	53	893.3
Average		5.9	5.3	89.33

isopropanol. The contents were mixed gently and collected by centrifugation at 4000 rpm for 10 min. The formed DNA pellet was washed twice with 70% ethanol and the ethanol was discarded after spinning with flash centrifugation. The remaining ethanol was removed by leaving the pellet to dry at room temperature. The pellet was dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8) and stored at -20°C for further use. The extracted DNA samples were observed under UV illumination after staining with ethidium bromide and agarose gel electrophoresis.

RAPD analysis and primer selection

A total of twenty five primers were screened using a few DNA samples to select the appropriate primers suitable for maize studies. Eventually, ten primers that produced strongly amplified polymorphic bands with these test templates were selected for RAPD-PCR analysis (Table 2). The PCR reaction was conducted in 50 µl reaction volume containing 1x PCR buffer, 1.5 mM MgCl , 0.2 mM of each dNTPs, 1 mM of primer, 1 U Taq DNA (promega) polymerase and 10 ng genomic DNA. DNA amplification was performed using a thermal cycler programmed for first cycle of 5 min at 94°C (initial strand separation); followed by 40 cycles of 1 min at 94°C (denaturation), 1 min at 60°C (annealing) and 2 min at 72°C and a final cycle at 72°C for 10 min. The PCR product were mixed with 2.5 µl of 10 X loading dye (0.25% bromophenol blue, 0.25% xylene cyanol and 40% sucrose, w/v) and spun briefly in a microfuge before loading. The PCR products and 1 kb DNA ladder were electrophoresed on 2% agarose gel at 100 V followed by staining with ethidium bromide and photographed on Polaroid 667 film under ultra-violet light.

Data analysis

The experiments were repeated for a minimum of three times to confirm the banding patterns and only those consistent bands on the gels were scored for data analysis. For each primer, the number of polymorphic and monomorphic bands was determined. Bands clearly visible in at least one genotype were scored (1) for present and (0) for absent, and entered into a data matrix. Fragment size was estimated by interpolation from the migration distance of marker fragments. Percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. The genetic dissimilarity (D) matrix among genotypes was estimated according to (Nei and Li, 1979). The similarity coefficient was used to construct a dendrogram by the unweighted pair group method with arithmetic averages (UPGMA) according to Rohlf (1993).

RESULTS AND DISCUSSION

In this study, 10 primers were used to produce a total of 59 amplified fragments-bands (Table 2) with average of 5.9 bands per primer, 53 of them were polymorphic (5.3 bands per primer) and 6 were monomorhic (0.6 bands per primer). The number of polymorphic bands varied from 3 for the OPA-20 primer up to 10 for the A-1 and C-2 primers. An example of the electrophoretic pattern of RAPD fragments, amplified from the UBC_{155} and C_2 primer is presented in Figure 1. The level of polymorphism (89.33%) obtained was higher than in some previous maize studies, such as Melo et al. (2001), who obtained 61.46% of polymorphic bands working with hybrids, and Lanza et al. (1997), who obtained 80.6% of polymorphism, studying genetic divergence between inbred lines using RAPD markers. One aspect to be considered is that in this study, taking into account the number and quality of the amplification products, the primers used were rigorously pre-selected, which might have contributed to increase in the level of polymorphism. The most important fact that should be taken into consideration is that the variations found in the level of polymorphism could be the result of the distinct regions in the maize genome that were assessed by the selected markers and/or of genotype differences between the

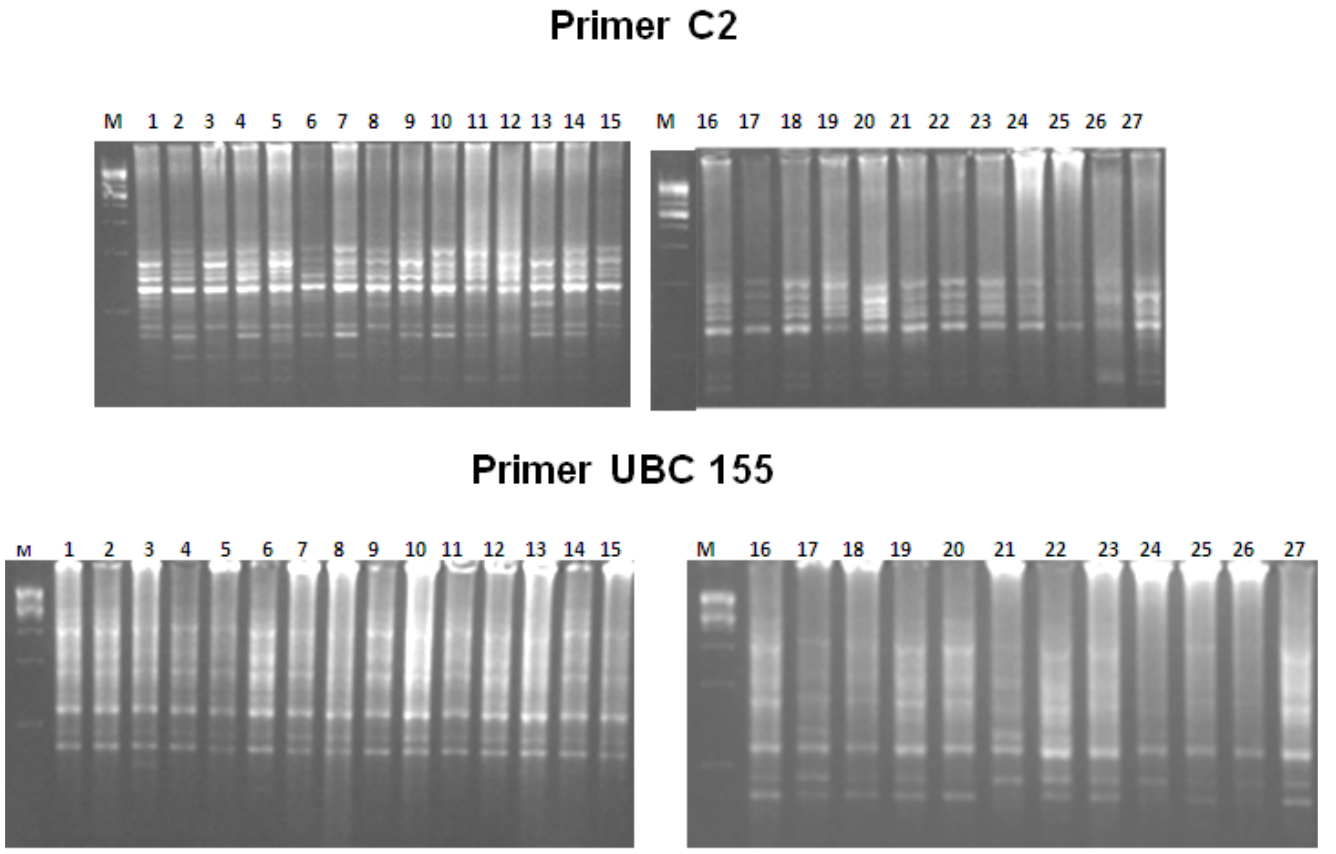

Figure 1. Photo of agarose gel (1.2%) showing amplified DNA fragments in the 16 lines analyzed with primers UBC_{155} and C_2, respectively (from left to right. Genotypes 1 to 27 and M- ladder 1 kb).

material used (Sun et al., 2001). The genetic similarity matrix of RAPD data for the 27 maize genotypes was constructed based on Nei and Li, (1979) coefficient of similarity shown in Table 3. The genetic similarities among all the possible pairs for the 27 maize genotypes ranged from 0.05 to 0.52. The value found in this study was a little above the values previously obtained by other authors dealing with maize. Sun et al. *(*2001) evaluated commercial hybrids in Canada and found a correlation of 0.43 using the RAPD and microsatellites. Laborda et al. (2005) worked with the AFLP and SSR markers in the tropical corn lines and obtained correlations of 0.43 and 0.48 using Jaccard's and Rogers' coefficients, respectively. Figure 2 presents the cluster for genotypes, according to the UPGMA clustering method, which groups genotypes by means of a dendrogram. The 27 genotypes were separated into three distinct clusters, the first one of which composed of six subclusters. The first subcluster in addition to inbred lines 160y and 66 as sisters also grouped inbred line 405 which was genetically distant from them. The second subcluster contained the open pollinated genotype Huediba 2, and the hybrid 66×3 which showed closeness. The third subcluster contained hybrids 160×3 and 405×160 as sisters. The fourth subcluster grouped hybrids 66×3 and 66×6 as sisters and hybrids 160×2 and 160×6 which showed genetic closeness. The fifth subcluster contained the inbred line 2 and hybrid 66×405. The sixth subclusters, grouped hybrids 3×405 and 66×160 which showed closeness. The second main cluster contained hybrids 66×277 and 3×2 which showed closeness. However, the third main cluster contained hybrids 2×277 and 6×2 as sisters which were genetically distant from all other genotypes. These results are in agreement with the heterotic patterns described by Lanza et al. (1997) who described that RAPD markers are useful to establish consistent heterotic groups between corn lines.

In conclusion, genetic diversity plays a key role in crop improvement. This study was aimed at identifying genetic diversity in 27 maize genotypes using 10 primer sets. Range of amplified fragments was from <250 to 750 bp in size. The degree of genetic polymorphism ranged from 0 to 100%, indicating that these genotypes were genetically very diverse and possesses a high amount of polymorphism. Average genetic distances ranged from 0 to 56%. It is recommended that these genotypes should be used in hybridization programs aimed at increasing level of genetic polymorphism in maize genotypes.

Table 3. Matrix of RAPD dissimilarity among 27 *maize (Zea mays* L.) genotypes based on coefficient was used to construct a dendrogram by unweighted pair group method with arithmetic average (UPGMA) according to Rohlf (1993).

S\N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	0.00																										
2	0.28	0.00																									
3	028	0.17	0.00																								
4	0.31	0.10	0.21	0.00																							
5	0.29	0.12	0.22	0.05	0.00																						
6	0.33	0.22	0.29	0.22	0.21	0.00																					
7	0.22	0.12	0.22	0.12	0.10	0.17	0.00																				
8	0.24	0.14	0.17	0.14	0.19	0.26	0.16	0.00																			
9	0.31	0.21	0.24	0.14	0.16	0.26	0.12	0.17	0.00																		
10	0.33	0.19	0.22	0.12	0.14	0.28	0.17	0.19	0.12	0.00																	
11	0.28	0.21	0.24	0.14	0.09	0.26	0.16	0.17	0.14	0.16	0.00																
12	0.31	0.28	0.34	0.24	0.22	0.19	0.22	0.24	0.17	0.22	0.17	0.00															
13	0.31	0.24	0.28	0.21	0.19	0.26	0.22	0.24	0.21	0.16	0.21	0.28	0.00														
14	0.34	0.24	0.28	0.17	0.19	0.33	0.22	0.24	0.17	0.26	0.21	0.31	0.21	0.00													
15	0.29	0.29	0.29	0.26	0.24	0.31	0.31	0.26	0.29	0.31	0.22	0.29	0.22	0.16	0.00												
16	0.24	0.21	0.24	0.17	0.19	0.29	0.19	0.17	0.21	0.26	0.21	0.28	0.31	0.31	0.26	0.00											
17	0.38	0.21	0.28	0.17	0.16	0.33	0.26	0.24	0.28	0.26	0.21	0.28	0.31	0.28	0.26	0.21	0.00										
18	0.31	0.17	0.21	0.17	0.16	0.29	0.19	0.24	0.21	0.19	0.21	0.24	0.24	0.28	0.26	0.21	0.21	0.00									
19	0.33	0.22	0.26	0.26	0.24	0.28	0.24	0.26	0.29	0.28	0.26	0.26	0.29	0.33	0.24	0.19	0.16	0.19	0.00								
20	0.29	0.26	0.22	0.19	0.21	0.24	0.21	0.19	0.22	0.24	0.22	0.29	0.29	0.29	0.31	0.22	0.22	0.22	0.21	0.00							
21	0.34	0.24	0.34	0.24	0.26	0.26	0.22	0.28	0.28	0.29	0.31	0.24	0.34	0.38	0.36	0.28	0.28	0.21	0.26	0.29	0.00						
22	0.28	0.24	0.21	0.17	0.19	0.26	0.19	0.21	0.21	0.22	0.17	0.24	0.28	0.28	0.19	0.14	0.21	0.17	0.16	0.12	0.24	0.00					
23	0.40	0.22	0.29	0.19	0.17	0.34	0.28	0.29	0.29	0.28	0.22	0.29	0.29	0.33	0.28	0.19	0.12	0.19	0.17	0.24	0.29	0.16	0.00				
24	0.52	0.41	0.41	0.41	0.40	0.47	0.43	0.48	0.41	0.40	0.41	0.31	0.38	0.48	0.47	0.38	0.34	0.28	0.33	0.43	0.21	0.34	0.26	0.00			
25	0.36	0.26	0.22	0.26	0.24	0.38	0.24	0.29	0.22	0.24	0.29	0.29	0.29	0.29	0.38	0.26	0.26	0.16	0.28	0.28	0.33	0.26	0.24	0.26	0.00		
26	0.48	0.34	0.38	0.31	0.29	0.40	0.36	0.38	0.31	0.36	0.34	0.38	0.34	0.28	0.33	0.31	0.28	0.28	0.33	0.36	0.34	0.34	0.33	0.31	0.22	0.00	
27	0.45	0.28	0.24	0.31	0.33	0.47	0.36	0.28	0.31	0.29	0.34	0.45	0.34	0.31	0.43	0.34	0.31	0.28	0.40	0.36	0.45	0.38	0.33	0.41	0.26	0.28	0.00

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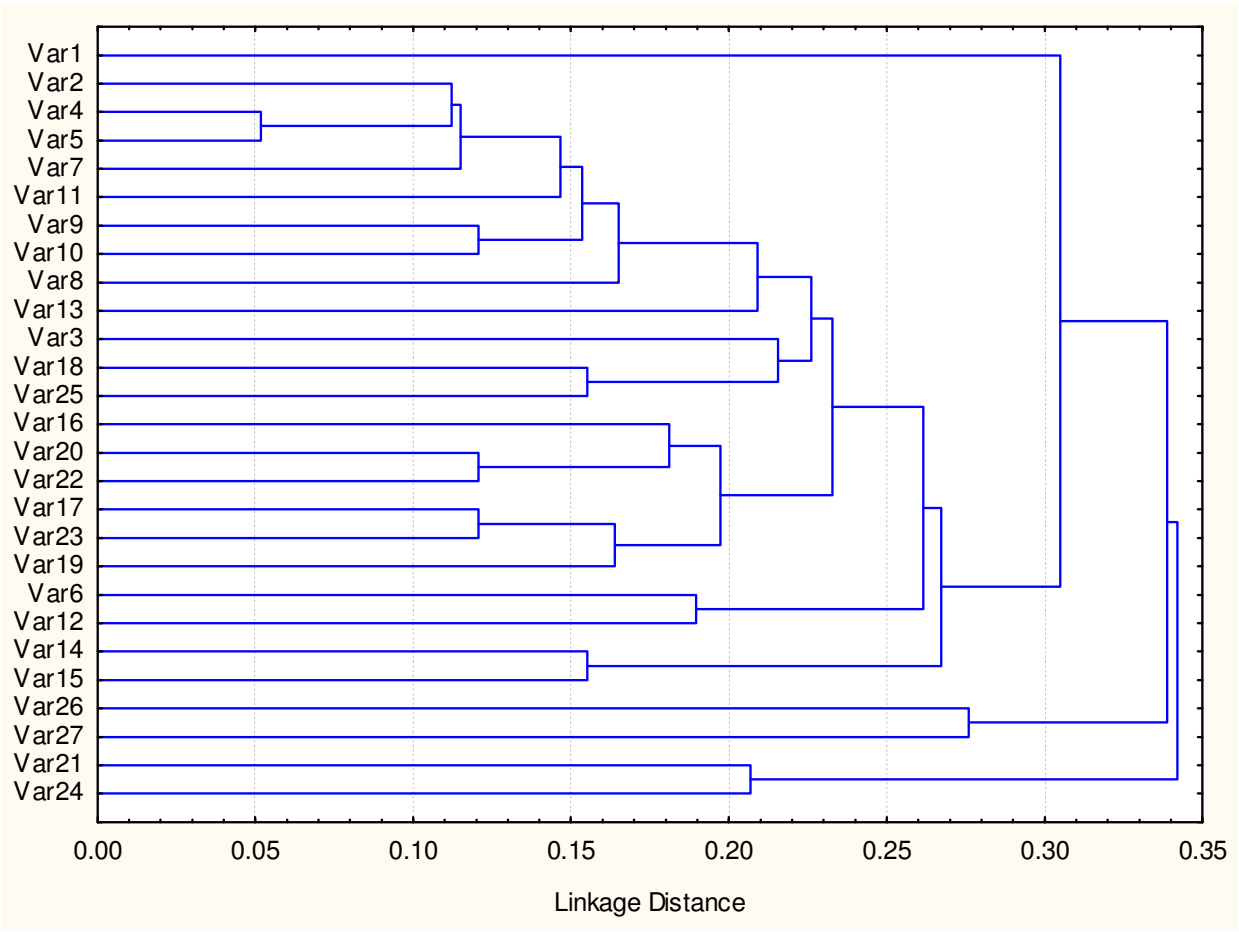

Figure 2. Dendrogram constructed for 27 *Z. mays* L. Genotypes based on genetic distances using 10 RAPD primers.

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