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Determining genetic distance by RAPD-PCR of maize inbred lines produced by reciprocal recurrent selection

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A field experiment was conducted at field crops station in Abu-Ghraib to produce maize inbred lines by reciprocal recurrent selection (RRS) for six seasons. A lab experiment followed to determine genetic variation among seven maize inbred lines tested for drought tolerance. Random Amplified Polymorphic DNA (RAPD) revealed high levels of polymorphism among inbred lines, where the percentage of polymorphism ranged from 93% to 42%. The highest number of polymorphic bands (15) gave with primer B04 while the primer O16 gave the lowest number of polymorphic bands (5), and the size of DNA fragments ranged between 4000 bp with primer H07 and 190 bp with primer B04. The genetic distance values ranged between 0.167 and 0.777, where the lowest genetic distance was between inbred lines (N4 and N9) while the highest genetic distance was between inbred lines (N4 and M13). Cluster analysis grouped the seven maize inbred lines into three groups based on genetic and morphological traits. We conclude that the results of RAPD-PCR are potentially extremely helpful in determining the genetic distance among maize inbred lines and this leads to election the inbred lines that may be used in the production of hybrids with high yield and drought tolerance depending on hybridization among them.

Keywords: DNA, RAPD, genetic diversity, maize inbred lines.

Introduction

Maize is the most important crops in the world; it is used mainly for human food, animal feed and industry (Moreno *et al.*, 2005). The improvement of maize traits increased by parents selection such as inbred lines and hybridization among them to produce hybrids that have high hybrid vigor (Vasal, 1992). The reciprocal recurrent selection has provided high efficiency in development of genetic population by systematic exploitation hybrid vigor that

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increases grain selection between heterogenetic group and within group (Xia *et al.,* 2005).

The main characteristics of genetic distance of inbred lines, which derived from heterogeneous, will increase the hybrids efficiency to tolerate stress condition (Xia *et al.,* 2005). It is not possible to predicate hybrids performance from inbred lines because of the high level of dominance for grain yield traits. Traditionally, genetic distance analysis of maize inbred lines was based on morphological traits while the recent studies have addressed molecular genetic markers. The use of molecular genetic markers is recently preferred as a feasible alternative approach to estimate genetic distance because the traditional breeding methods are expensive, time consuming, and affected by the environment (Joyce *et al.*, 1999).

The development of modern maize breeding programs has been depended on polymerase chain reaction (PCR) based techniques such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR) (Saiki *et al*., 1988; Laborda *et al.,* 2005; Zand and Gavanji, 2013). These techniques used to determinate the genetic diversity analysis, and comparison among plant populations for several plant species such as maize (Marsan *et al*., 1993; valdmer *et al*., 2004; Molin *et al.*, 2013), rice (Younan *et al.,* 2011), alfalfa (Yu and Palus, 1993), rose (Zand and Gavanji, 2013), and others.

One of the most technique that widely used to determine the genetic diversity is Random amplified polymorphic DNA (RAPD) because of its low cost and its simplicity, as well as it allows to detect the polymorphism rapidly(Wu, 2000; Aslam *et al.,* 2009; Molin *et al.,* 2013). RAPD-PCR generates several DNA fragments, with different sizes as a result of using single random primer, which consisting of ten nucleotides with a random sequence (Ribaul *et al*., 1999; Souza *et al*., 2008). The results of this technique can be analyzed by using gel electrophoresis, and then determined the polymorphism in the DNA sequence of the tested samples from the set of DNA fragments that generated (Williams *et al*., 1990; Moeller and Schaal, 1999).

The objective of this study is investigating the genetic distance and constructing the genetic relationship among maize inbred lines for exploiting the genetic variations in the future programs of maize breeding.

Material and methods

Plant Materials

Seven maize inbred lines produced by reciprocal recurrent selection (RRS) evaluated in this study (M8, M9, M12, M13, N4, N5, and N9). These inbred lines were grown by two periods of irrigation (5 and 10 days) in order to assess their efficiency under drought stress by measuring some traits (Table 1).

DNA Isolation

Total genomic DNA extracted from dry seed using extraction kit, wizard genomic DNA purification kit, provided by Promega, USA. The integrity of DNA confirmed by using 0.8% agarose gel electrophoresis followed by staining with ethidium bromide (Maniatis *et al.*, 1982). The yields and purity of DNA was determined by spectrophotometer at wave length 260-280 nm (Sambrook *et al.*, 1989).

RAPD-PCR analyses

Nine tenmers of oligonucleotide that provided by Alpha DNA company (Canada) were used. The primers are A08, A13, B01, B04, C05, D20, O16, H07 and R02, the details of these primers shown in table (2). RAPD-PCR reactions were achieved with the final volume of 25 µL, containing 12.5 µL of (2X) Go Taq ® Green Master Mix (Promega, USA); 1 µL of (10 pmol/µL) primer; 2 µL of (50 ng/µL) DNA template and 9.5 µL of free nuclease distilled water. All the PCR reactions were conducted by using thermalcycler (MultigeneTM Gradient Thermal Cycler, Labnet International, Korea) under the following conditions: in order to initial denaturation, just one cycle at 94^{o}C for 5 min., followed by 45 cycles of denaturation for 1 min. at 94^{o}C; annealing of primer for 1 min. at 36^{o}C; primer extension for 2 min. at 72^{o}C, after that one cycle at 72^{o}C for 10 min. as a final extension. PCR products were running in 1.5% agarose gels along with 1 Kb DNA ladder (Promega, USA). After gel electrophoresis, agarose gel stained with ethidium bromide and this gel was captured using gel documentation system (E-graph, Korea).

Molecular Weight Estimation

Photo-capture M.W program (Consort-Belgium), computer software M.W detection program, was used to estimate the molecular weight (M.W) of PCR

products by comparing with the known size of DNA fragments of a DNA ladder.

Genetic Distance Estimation and Cluster analysis

The genetic distances were estimated upon Nei and Li, (1979) by comparing the amplification profile of all inbred lines in the experiments for each primer with others, where consist table known (table 0,1). The presence of band scored as "1" and the absence of the same band of the same size in other inbred lines as "0".

G.D = 1- [2 Nab / (Na + Nb)]

Where

Na = the total number of fragments detected in individual "a".

Nb = the total number of fragments shown by individual "b".

Nab = the number of fragment participated by individual "a" and "b".

Cluster analysis was performed to construct genetic relationship tree diagram, with inbred lines of maize using an Uweighted Pair-Group Method with Arithmetic Average (UPGMA) (Rohlf, 1993; Younan *et al.*, 2011).

Results and discussions

Results of RAPD-PCR technique

RAPD-PCR technique used to determined the genetic distance among seven maize inbred lines. The total number of main bands that were produced by nine random primers was 117 bands, involving 33 monomorphic bands with percentage 28.2% while the other bands (84 bands or 71.8%) were polymorphic. The number of main bands was higher than that found by Aslam *et al.* (2009) study, which assessed 10 maize accessions using 25 primers, while it was lower than that found by Carvalho *et al.* (2004), who studied genetic diversity among 81 maize landraces using 32 highly informative primers.

The number of polymorphic bands ranged between 5-15 bands; the primer B04 produced the highest number of polymorphic bands amount to 15 bands while the primers O16 gave the lowest number of polymorphic bands amount to 5 bands, the average of polymorphic bands equal to 9.33 bands per primers among seven maize inbred lines. In the other studies the average of polymorphic bands was either a little above or a little under than that found in this study as reported in the following studies: Souza *et al.* (2008) and Carvalho

et al. (2004), respectively. The size of DNA fragments ranged from 4000 bp for primer H07 to 190 bp for primer B04.

The highest percentage of polymorphism was 93% obtained by the primer B01 while the lowest percentage was 42% produced by O16 (Table 2). Figure (1) show the results of two primers, primer C05 that appeared high polymorphism, but primer D20 was appeared low polymorphism

Table 1. Some agronomic traits of inbred lines

Inbred lines	Leaf area (m^2)	Grain weight (mg)	Yield ($ton.ha^{-1}$)
M_8	0.42	278.66	4.89
M_9	0.39	273.66	3.09
M_{12}	0.38	260.66	4.48
M_{13}	0.31	243.00	4.60
N_4	0.33	271.66	4.69
N_5	0.35	279.66	4.18
N_9	0.37	280.00	4.87

Table 2. Primers and their sequences, number of main and polymorphic bands and their percentage of polymorphism across the inbred lines of maize

No.	Primer	Sequence (5'~3')	Number of main bands	Number of polymorphic bands	Polymorphism %
1	A08	GTGACGTAGG	12	10	83
2	A13	CAGCACCCAC	16	8	50
3	B01	GTTTCGCTCC	15	14	93
4	B04	GGACTGGAGT	18	15	83
5	C05	GATGACCGCC	9	8	89
6	D20	ACCCGGTCAC	11	6	54.5
7	H07	CTGCATCGTG	11	10	91
8	O16	TCGGCGGTTC	12	5	42
9	R02	CACAGCTGCC	13	8	61.5
Total			117	84	___

Genetic distance

Genetic distance is any quantitative measure of genetic difference, be it at the sequence level or the allelic frequency level that calculated between individuals, populations or species (Beaumont *et al.*, 1998). The genetic distance values ranged from 0.16764 to 0.77760 as shown in table (3). The lowest genetic distance value (16.7%) was obtained between inbred line C7 (N9) and C5 (N4) while the highest genetic distance (77.7%) came from inbred line C5 (N4) and C4 (M13). Overall, the genetic distance results show high

similarity in genetic materials of N9 and N4, which was 83.24%, this results parallel to results of the other studies such as Aslam *et al.* (2009) and Souza *et al.*, (2008). On the other hand, the low similarity in genetic materials of N4 and N13 (22.2%) was lowest among than the lower similarity that resulted across different maize lines (*Zea mays*) (Carvalho *et al.*, 2004; Souza *et al.*, 2008; Aslam *et al.*, 2009; Molin *et al.*, 2013).

Table 3. Values of genetic distances among seven maize inbred lines calculated according to Nei and Li (1979) (data matrix). Samples C (1-7): M8, M9, M12, M13, N4, N5, and N9, respectively

	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**
C1	0.00000						
C2	0.29599	0.00000					
C3	0.45413	0.30896	0.00000				
C4	0.35266	0.50295	0.65486	0.00000			
C5	0.43141	0.38446	0.54259	0.77760	0.00000		
C6	0.37396	0.37010	0.37408	0.66566	0.46242	0.00000	
C7	0.41565	0.35117	0.41577	0.67098	0.16764	0.36377	0.00000

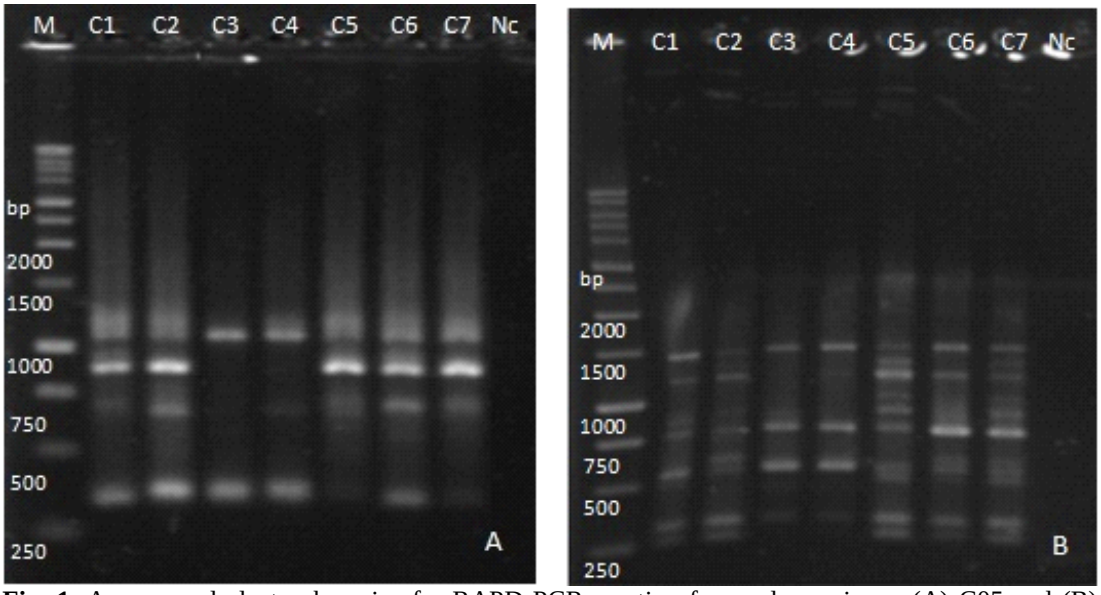

Fig. 1. Agarose gel electrophoresis of a RAPD-PCR reaction for random primers (A) C05 and (B) D20 for DNA samples of the inbred lines (under optimal condition). Bands fractionated by electrophoresis on 1.5% agarose gel (2hr, 5V/cm, and 0.5XTris -borate buffer) and visualized

Cluster analysis

Based on genetic distance formula of Nei and Li (1979) and using UPGMA cluster analysis program the genetic relationship among seven maize inbred lines was constructed and depicted. The dendrogram tree was shown that there were three major clusters (Figure 2). The first cluster was C4 (M13) that occurred as a separate cluster from the other inbred lines in this study because it had the greatest genetically distance. The second cluster formed from the inbred lines C2 (M9) and C1 (M8) as a sub- cluster; also, the inbred lines C6 (N5) and C3 (M12) occurred within this cluster. The third cluster was formed from the inbred lines C7 (N9) and C5 (N4) that have the highest genetic similarity comparing with the other inbred lines, also these inbreeds C1 (M8) and C2 (M9) gave similarity with morphological traits such as leaf area (m^2) 0.42 and 0.39 sequencely, else inbreeds C7 (N9) and C5 (N4) appear similarity with yield ton ha^{-1} 4.69 and 4.87 respectively, where these results similar with cluster analysis. The results revealed that maize inbred line C4 (M13) had big genetic distance values from the other and constructed a separated cluster; therefore it can be used in hybridization program for exploiting hybrid vigor phenomena, especially on crossing with inbred line genetically high distance such as C5 (N4) or C7 (N9) that formed the other cluster. On the other hand, the genetic distance value between C7 (N9) and C5 (N4) was so low; therefore, no hybrid vigor is predicted from crossing between them.

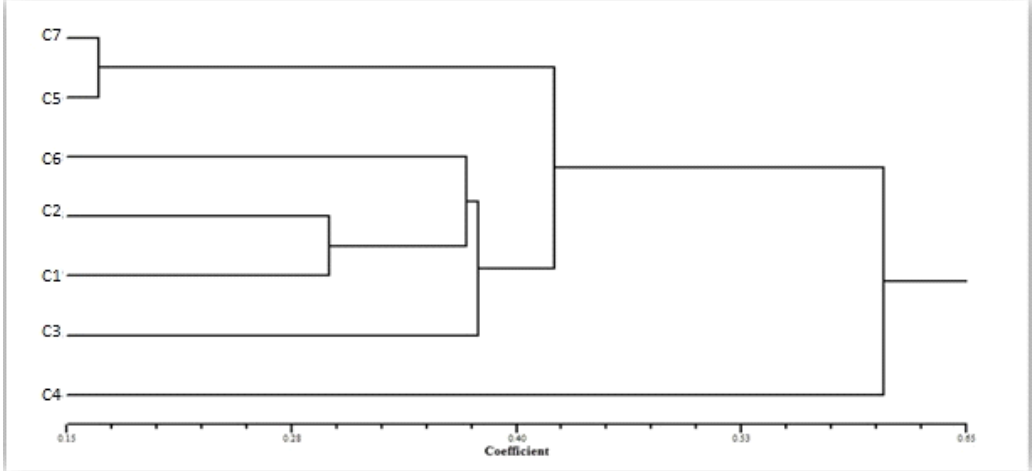

Fig. 2. Dendrogram of seven maize inbred lines produced using UPGMA cluster analysis based on the genetic distance matrix.

Conclusion

We conclude that the results of RAPD-PCR are extremely useful in determining the genetic distance among maize inbred lines, and this leads to election the inbred lines that may be used in the production of hybrids with desired traits such as high yield and drought tolerance, where this research provides insight for further studies on breeding programs for maize inbred lines.

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