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Assessment genetic diversity among some Egyptian bread wheat (*Triticum aestivum* L.) cultivars using RAPD markers

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Abstract

Genetic diversity was investigated among seven bread wheat varieties originated from Egypt by 17 RAPD markers and twelve agronomic traits. The RAPD markers were capable of detecting 62 alleles with an average of 3.64 alleles per marker. The number of alleles per marker ranged from 3 to 6 and the allelic polymorphism information content (PIC) value ranged from 0.571 for the *OPA-07*, *OPO-03* and *OPO-12* to 0.816 for the *OPA-01* with an average of 0.681. The results revealed that the varieties differed for RAPD markers and agronomic traits. The significant correlation coefficient between gene diversity and the number of polymorphic bands was high, r = 0.733 (P <0.01). Cluster analysis was conducted based on RAPD to group the wheat varieties and construct a dendrogram. Three main groups were distinguished by RAPD. RAPD markers showed a high level of polymorphism among the varieties examined. The present study indicates that RAPD markers could be successfully used in genetic characterization and diversity in wheat. Also, Information generated from this study can be used to select parents for hybrid development to maximize yield and its components.

Keywords: Bread wheat (*Triticum aestivum* L.), RAPD markers, Genetic diversity, Polymorphism information content (PIC).

Introduction

Genus *Triticum* consists of various species, which include diploids (2n = 2x = 14), tetraploids (2n = 4x = 28) and hexaploids (2n = 6x = 42). Bread wheat (*Triticum aestivum* L.), encompasses three different other than genetically related genomes (A, B and D) with a total genomic size of 1.7×10^{10} base pairs, which is about 500 fold greater than that of rice. Moore *et al.* (1995) demonstrating the complex nature of the wheat genome. The total number of accessions of wheat in international and local gene banks around the world is estimated to be more than 400,000, although many accessions may be duplicated in different collections (Poelham and Sleper 1995). The study of genetic diversity is important in a crop breeding program for the selection of suitably diverse parents to obtain heterotic hybrids as well as for the conservation and characterization of germplasm (El-Absawy et al. 2015; Salem and Sallam, 2016). Assessment of crop germplasm phenotypically and morphologically is usually devoid of the resolving power needed to identify an individual genotype. Estimation by biochemical markers, viz. isozyme analysis, may also be biased as only a minor portion of the genome is represented by these markers (Second 1982). In the last decade, molecular markers, such as RFLP, RAPD, SCAR, AFLP, SSRs, SNPs and ESTs have been used to assess genetic variations at the DNA level (Salem et al. 2007; Salem et al., 2008; Varshney et al. 2008; Salem 2009; El-Zanaty, et al. 2011; El Rabey et al. 2013; Salem and Mattar 2014a,b; Salem 2015; Mattar et al. 2016; Mourad et al. 2019). Molecular markers are a useful complement to the morphological and physiological characterization of cultivars because they are plentiful, independent of tissue or environmental effects and allow cultivar identification in the earlier stages of plant development (Miller and Tanksley 1990). Techniques using isozyme and RFLP are time-consuming, labor-intensive and reveal a low level of polymorphism in wheat owing to its high proportion of repetitive DNA. Random amplified polymorphic DNA (RAPD) markers (Williams *et al*, 1990, Welsh and McClelland 1990), on the other hand, are an effective method for detecting polymorphism in wheat (Sun *et al*, 1998, Eid 2019) and various other crops (Virk et al 1995, Penner 1996, Sadeghi and Cheghamirza 2012). Factors such as speed, efficiency and amenability to automation make RAPD one of the most suitable methods for germplasm management to

estimating diversity, monitoring genetic erosion and removing duplicates from germplasm collections (Penner 1996). The present study has, therefore, been undertaken to i) use RAPD and agronomic traits to assess genetic diversity among some bread wheat varieties, ii) analyze the genetic relationships among them for future studies on the evaluation and conservation of wheat genetic resources.

Material and Methods

Plant materials: Seven wheat genotypes were used in this study i.e., Giza 168, Gemmiza-7, Gemmiza-9, Gemmiza-10, Gemmiza-11, Sids 12 and Shandaweel-1 as local genotypes from Agriculture Research Center (ARC), Giza, Egypt and these varieties were withdrawn from the genetic stock of wheat section, Field Crops Research Institute, ARC, Egypt. The name, origin and pedigree of the seven wheat genotypes are presented in (Table 1).

Table 1. Wheat varieties, origin, and pedigree used in the study.

No.	Varity	Pedigree	Selection history	Year of release
1	Giza-168	MIL/BUC// Seri CM93046-8M-0Y-0M-2Y-0M	CM93046-8M-0Y-0M-2Y-2B	1999
2	Gemmiza-7	CMH74A.360/5x//Seri82/3/Agent CGM4611-2GM-3GM-1GM-0GM.	CGM 4611-2GM-3GM-1GM-0GM	2000
3	Gemmiza-9	ALD”S”/HUAC”S”//CMH74A.630/5X.	CGM 4583-5GM-1GM-0GM	2000
4	Gemmiza-10	MAYA74"S"/ON//II60.147/3/BB/GLL/4/CHAT"S" /5/CROW"S"		2004
5	Gemmiza-11	BUC’’S’’/ Kvz’’S’’// 7c/ Seri 82 /3/Giza 168/ Sakha 61 GM7892-2GM-1GM- 0 GM.		2011
6	Sids-12	BUC// 7c/ Ald/5/ Maya 74/ On/ 1160.147/3/ BB/ G11/4/ Chat’’S’’ /6/ Maya/ vu1 // Cmh 74A.630/4* sx, SD7096- 4SD- 1SD-0SD.	SD7096-4SD-1SD-1SD-0SD	2008
7	Shandaweel-1	Site//Mo/4/Nac/Th.Ac./3*Pvn/3/Mirlo/Buc.	CMSS93B00567S-72Y-010M-010Y-010M-0HTY	2013

Genomic DNA extraction: Genomic DNA extraction was performed according to a method described by Salem (2004) as follows: leaves of 15 days old (~100 mg) of the seven wheat varieties were placed separately in 1.5 E-cup centrifuge tubes and ground by using a plastic pistol and mortar. 400 µl of extraction buffer (200 mM Tris/HCl pH 7.5, 250 mM NaCl, 25 mM EDTA and 0.5% SDS) were added and incubated at 65 °C for 30 min. The same volume of chloroform was added, vortexed for 5 sec, and centrifuged at 13000 rpm for 5 min. The upper phase ~ 300 µl was transferred to a new tube, the same volume of cooled isopropanol was added, carefully mixed, incubated 2 min at room temperature and centrifuged at 10000 rpm for 5 min. Pellet was washed in 500 µl of 70% ethanol by centrifuging at 13000 rpm for 5 min, the supernatant was discarded and the pellet was left for air dry. Then the pellet was resuspended in 200 µl of TE buffer pH 8.0 and incubated at 65 °C until dissolved (1-4 h), then incubated at 4 °C for continued dissolution. To check gel control 5 µl were loaded on 1% TAE electrophoresis gel and 1 µl was used as a template for PCR reaction.

Determination of DNA concentration: DNA quantity and purity were estimated using a Nanodrop device at 260/280 nm in order to determine DNA concentration. The integrity of DNA samples was assessed by loading 5 µl of DNA samples on 1% agarose gel and visualized under UV light using the gel documentation system.

RAPD Marker analysis: PCR amplification was used with 17 RAPD primers (Operon Technologies, Alameda, USA); The RAPD primers and their sequences are presented in Table (2), which was performed using a TECHNE-TC-4000 thermocycler (Bibby Scientific Ltd, Inc., United Kingdom). A total of 20 µL PCR reaction mixture contained 10 µL of 2x PCR Master Mix solution (i-Taq), 2 µL of 0.25 µ*M* of primer, 2 µL of 25 ng/µl templates DNA and 6 µL H_2O. PCR amplification was performed with a hot start of 94 ºC (4 min) followed by 35 cycles of denaturation at 94 ^oC for 1 min, annealing at 29-34 ^oC for 1 min and extension at 72 ºC for 1 min. The final extension was carried out at 72 ^oC for 7 min (Salem 2015). Amplified DNA fragments were separated by 1% agarose gel electrophoresis in Tris-Borate EDTA buffer. The size (bp) of the

uppermost and the lowermost band for each RAPD primer was determined based on its relative migration to the molecular weight size 50 bp DNA ladder. The DNA bands were made visual by staining the gels with ethidium bromide and were photographed under UV light using the gel documentation system.

Band Scoring and Data Analysis: The frequency of RAPD polymorphism between 7 wheat genotypes was calculated based on the presence of the band taken as '1' or absence of band taken as '0'. Genetic similarity coefficients were calculated using the Numerical Taxonomy Multivariate Analysis System (NTSYSpc) Version 2.1 software package. The resulting similarity coefficients were used to perform the cluster analysis by the unweighted pair group method of the arithmetic mean (UPGMA). All calculations were performed using the NTSYS-pc version 2.1 software package Biostatistics Inc., USA, (Rohlf, 2000). RAPD polymorphism rates were determined using polymorphism information content (PIC) value, which was calculated according to the formula: $\mathrm{PIC} = 1 - \sum_{i=1}^{k} P_i^2$, where k is the total number of alleles detected for a marker of a marker and P*i* is the frequency of the *i*th allele in the set of seven genotypes investigated (Anderson *et al.,* 1993).

Table 2. Primer names and sequences of RAPD markers used in this study.

Primer number	Primer name	sequence '5-3'
1	OPA-01	CAGGCCCTTC
2	OPA-03	AGTCAGCCAC
3	OPA-05	AGGGGTCTTG
4	OPA-07	GAAACGGGTG
5	OPB-03	CATCCCCCTG
6	OPC-01	TTCGAGCCAG
7	OPD-03	GTCGCCGTCA
8	OPH-01	GGTCGGAGAA
9	OPH-03	AGACGTCCAC
10	OPO-01	GGCACGTAAG
11	OPO-02	ACGTAGCGTC
12	OPO-03	CTGTTGCTAC
13	OPO-11	GACAGGAGGT
14	OPO-12	CAGTGCTGTG
15	OPO-13	GTCAGAGTCC
16	OPO-14	AGCATGGCTC
17	OPO-15	TGGCGTCCTT

Results and Discussion

RAPD banding profile and polymorphism: Seventeen primers with ten base arbitrarily were amplifying the genomic DNA of seven wheat genotypes (Sids-12, Gemmiza-11, Gemmiza-10, Gemmiza-7, Gemmiza-9, Giza-168 and Shandaweel-1), produced 62 bands. The total number of DNA bands amplified (111) varied between 3 and 11 with an average of 6.53 bands per primer. The primer OPO-13 gave the highest number of bands (11), while the OPH-03 and OPO-11 primers yielded the lowest number of bands (3). The primer produced a higher number of bands that could have a better application to find out the polymorphisms in the heritability of wheat cultivars. It has been shown that increasing the number of alleles, indicating a greater magnitude of diversity among the plant materials included in this investigation. The level of variation depicted by the number of bands at each primer serves as a measure of genetic variability having a direct effect on the differentiation of cultivars within a species. The high mean number of observed alleles (allelic diversity) per primer displayed high genetic variation and indicates that the population is under mutation drift equilibrium (Salem and Sallam 2016). The total percentage of polymorphism for all primers used was 27.84%. This amply suggested that the genotypes selected for this study harbor enough genetic divergence. This value of polymorphism is constant partially with Ramiz et al. (2007). Out of 77 loci scored, the 21 were monomorphic, identical for all analyzed genotypes, may indicate that the primer has annealing sites at the same position of all genotypes genomes and/or maybe attributed to the amplification of highly conserved regions in the genome that make amplification bands similar to each other in

molecular weight (monomorphic) (Qadir et al. 2017). The percentage of polymorphic bands obtained for each primer did not correlate to the total number of bands. For instance, the total number of bands scored for primer OPO-02 and OPO-13 was relatively high, 10 and 11, respectively, with 50 and 63.64% of them being polymorphic. In contrast, the lowest number of total bands was obtained for OPO-11 and OPC-01 and both of these were polymorphic (OPO-11 and OPC-01 33.33 and 40%, respectively) (Table 3).

The variation in polymorphism among the primers in one cultivar or the all primers in one cultivar may be attributed to several causes such as the loss or alteration (deletion or insertion) of one or both of the opposed pair of primer binding sites needed to produce the PCR product or as a result of nucleotide changes (e.g. point mutation) at the primer annealing site in genome DNA that prevents amplification by introducing a mismatch at just one end of a DNA segment then the DNA polymorphism observed by RAPD results. Alternatively, an insertion between the primer annealing sites may place them at different distances (lengths) or even too great to allow the amplification products (Hurtado and Rodríguez-Valera 1999).

Gene diversity: The polymorphism information content is the probability of detection of polymorphism by a primer/primer combination between two randomly drawn genotypes and depends on the number of detectable alleles and the distribution of their frequency. Because of the high credibility of the PIC index, this parameter has been used widely in numerous genetic diversity researches (Najaphy et al. 2011, Sadeghi and CHeghamirza 2012, Safari et al. 2013). The average of PIC values for the seventeen primers was 0.681 and ranged from 0.571 for the OPA-07, OPO-03 and OPO-12 to 0.816 for the OPA-01 (Table 3). Lower PIC values may be the result of closely related genotypes and higher PIC values might be the result of diverse genotypes. All primers had PIC values above 0.5 indicating that these primers are highly informative for determining wheat genotypes polymorphism. According to Botstein et al. (1980) reported that molecular markers have PIC values higher than 0.5 are considered highly informative. These high estimates of PIC substantiated the suitability of a used set of DNA markers to applications such as linkage-mapping programs in addition, to genetic polymorphism studies in other wheat cultivars breeding too. Gene diversity obtained in the present investigation was comparable with previous results on the genetic diversity of wheat using RAPD analysis. In this result, the PIC values were found to be higher than that of Najaphy et al. (2011). It could be attributed to the diverse nature of the five wheat genotypes and/or highly informative eight RAPD markers used.

Table 3. Primer code, the total number of bands, number of polymorphic bands and percentage of Polymorphism.

Primer code	Total no. of bands	No. of polymorphic bands	Polymorphism %	Polymorphism information content
OPA-01	8	1	12.5	0.816
OPA-03	7	4	57.14	0.694
OPA-05	8	1	12.5	0.653
OPA-07	6	2	33.33	0.571
OPB-03	6	2	33.33	0.653
OPC-01	5	2	40	0.776
OPD-03	6	3	50	0.735
OPH-01	4	0	0	0
OPH-03	3	0	0	0
OPO-01	6	1	16.67	0.653
OPO-02	10	5	50	0.714
OPO-03	8	2	25	0.571
OPO-11	3	1	33.33	0.735
OPO-12	8	1	12.5	0.571
OPO-13	11	7	63.64	0.776
OPO-14	6	1	16.67	0.653
OPO-15	6	1	16.67	0.735
Total	111	34	-	-
Average	6.53	2	27.84	0.681

The present findings showed that the importance of gene diversity increased with the increasing number of alleles at a given locus (Fig. 1). The correlation coefficient between gene diversity and the number of polymorphic bands was high, $r = 0.733$ ($P < 0.01$). The linear relationship between them is shown in Figure 1. The number of alleles can also be used for genetic diversity assessment. Our findings are consistent with those reported by Huang et al. (2002) and Salem et al. (2010, 2015) that the PIC value was associated with the number of alleles and did not align with those reported by Prasad et al. (2000).

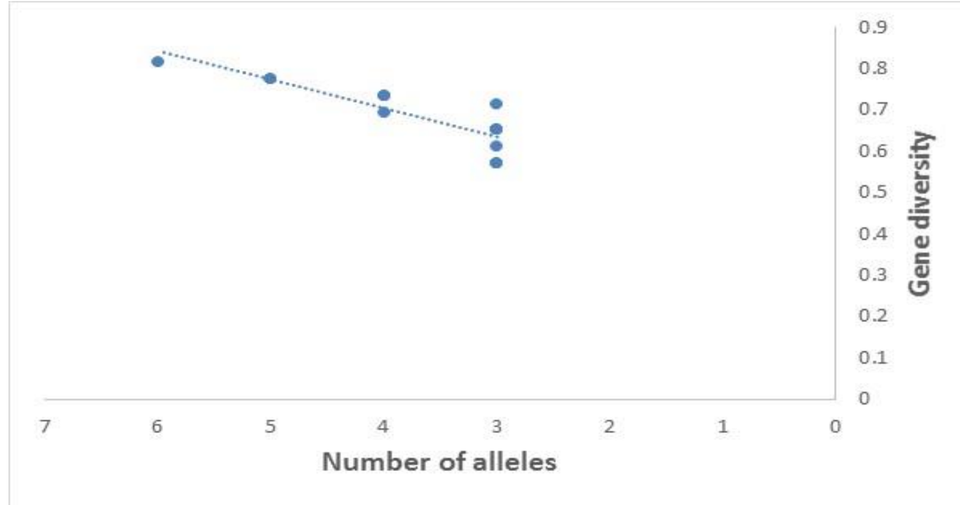

Figure 1: Correlation between gene diversity and the number of alleles detected at 17 RAPD loci. (y = 0.0694x + 0.4274, R^2 = 0.733)

Genetic relationship and diversity among different wheat varieties: To assess the genetic diversity of wheat varieties, marker data were converted into a binary matrix, which in turn allowed us to calculate the genetic similarity (GS) index. A dendrogram derived from UPGMA cluster analysis based on the GS coefficient matrix for the seven varieties was constructed. All varieties could be distinguished. The genetic similarity coefficient for all varieties ranged from 0.108 to 0.671 (Table 4).

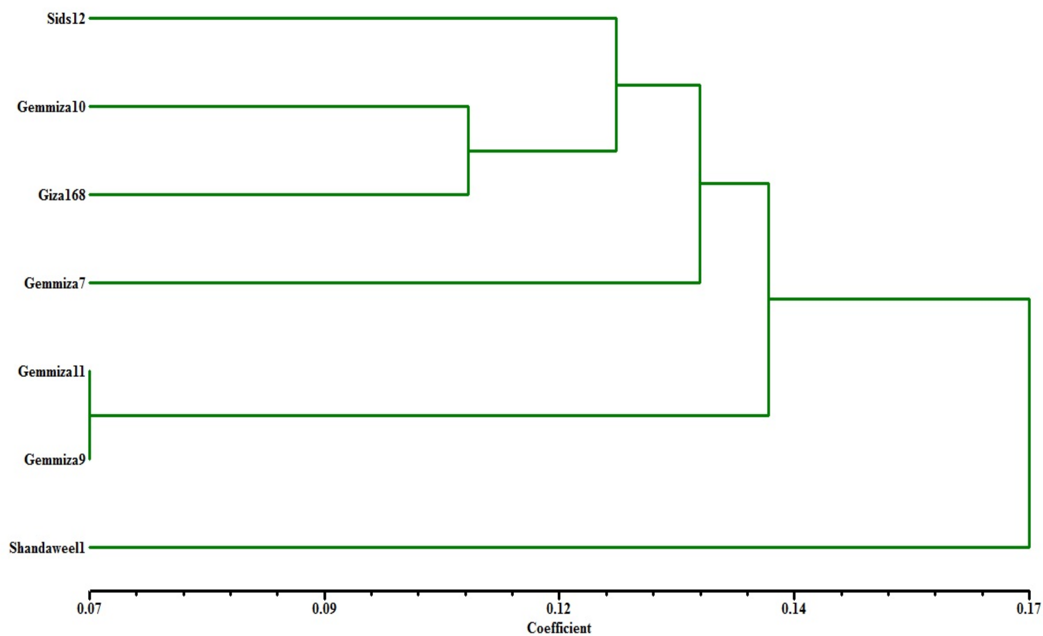

Fig. 2. Dendrogram generated based on UPGMA clustering using RAPD analysis among seven bread wheat genotypes.

Table 4. Genetic similarity estimates for seven wheat genotypes based on seventeen RAPD markers.

Genotypes	Sids 12	Gemmiza 11	Gemmiza 10	Gemmiza 7	Gemmiza 9	Giza 168	Shandaweel 1
Sids 12	1						
Gemmiza 11	0.119	1					
Gemmiza 10	0.119	0.119	1				
Gemmiza 7	0.152	0.152	0.129	1			
Gemmiza 9	0.152	0.671	0.129	0.141	1		
Giza 168	0.129	0.174	0.108	0.119	0.140	1	
Shandaweel 1	0.175	0.198	0.174	0.163	0.186	0.190	1

A dendrogram was created with the use of these data (Fig. 2) for each pairwise similarity estimation. The consensus tree showed that the seven wheat varieties were divided into three major groups. Group I consisted of four bread wheat varieties Sids 12, Gemmiza 10, Giza 168 and Gemmiza 7 and was divided into three subgroups. Subgroup IA consisted of one variety Sids 12. The subgroup IB included the two varieties Gemmiza 10 and Giza 168. However, The subgroup IC included only one variety Gemmiza 7. However, Group II consisted of two variety Gemmiza 11 and Gemmiza 9. However, group III consisted of only one variety Shandaweel 1. In general, the similarity indices showed that the two most closely related varieties were Gemmiza 11 and Gemmiza 9 (Fig 2). On the other hand, the two Egyptian wheat varieties Gemmiza 10 and Giza 168 were the most genetically diversified from other varieties sources and could be important sources for new cultivar development if they differ in useful agronomic traits. It should be noted here that varieties grouping here by cluster analysis depended on the polymorphic RAPD markers. Cultivars grouped together by the RAPD markers could have noticeable phenotypic differences in morphology, growth habits and agronomic traits. The knowledge about the genetic relationships of varieties offers valuable information to resolve the breeding program and germplasm resource management (Roldán-Ruiz et al., 2001).

Conclusion

In summary, among some Egyptian bread wheat genotypes, our data showed significant variation in RAPD polymorphisms. Among seven varieties of wheat, this study using RAPD markers showed a large amount of genetic diversity. RAPD data can be used to use the genetic potential of these varieties to improve the characteristics required for adaptation to various stress conditions while selecting different parents in the breeding program and maintaining genetic diversity in the germplasm. This study also shows that unless a particular crossing program is aimed at enhancing specific characteristics, comparing higher numbers of varieties may not give a general plant enhancement program much practical value. It is therefore suggested that when assessing genome diversity estimates for parent selection, and the oriented breeding scheme should be introduced to achieve maximum benefit and a practical impact on a breeding program.

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