Genetic Diversity of Some Egyptian Durum Wheat Cultivars

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Abstract: The objective of this investigation was to assess the genetic diversity among three Egyptian durum wheat i.e. Beni Suif 4, Beni Suif 5 and Beni Suif 6 and one bread wheat i.e. Sids 12 cultivars using sodium dodecyle sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and randomly amplified polymorphic (RAPD) markers. Protein electrophoreses showed that Beni Suif 4 was characterized by the absence of band-3 with 41.56 kDa. RAPD analysis showed that the number of polymorphic amplicons was 56 out of a total of 93 amplicons, thus revealing a level of 60.0 % polymorphism. The highest genetic similarity revealed by RAPD analysis (95.0%) was between Beni Suif 5 and Beni suif 6. While, lowest similarity (65 %) was found between Beni Suif 4 and Beni Suif 5. The dendrogram separated Sids 12 from all the other genotypes, thus demonstrating the distinctiveness of the genetic background of this genotype from all the other genotypes. The three genotypes constituted a subcluster divided into two groups, one group composed of Beni Suif 5 and Beni Suif 6 , while the second group comprised Beni Suif 4.
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Key words: Durum Wheat, RAPD, Dendrogram, Dice coefficient, Polymorphism, Turgidum.

1. Introduction:

Morphological characters have been used to identify plant species, families and varsities. However, they have many disadvantages, since they are influenced by the environment and scoring is a time-consuming process. The use of genetic molecular markers (protein and DNA-based) have become widely accepted valuable tools (Cooke, 1999). SDS-PAGE is considered as a low cost, reproducible and rapid approach (Laemmli, 1970). In the last decade, molecular markers such as RFLP, RAPD, ISSR, AFLP have been used to assess genetic variation at the DNA level, allowing an estimation of degree of relatedness between individuals without the influence of environmental variation (Gupta *et al*., 1999). DNA RAPD is a useful method for generating molecular markers (Welsh and McClelland, 1990) that can be used to construct linkage maps, to identify varieties (He *et al*.1992) and to assess genetic diversity (Koller *et al.,* 1993). It is characterized by its low technical input and small quantity of plant DNA needed for the analysis (Hernandez *et al.*, 1999 and Manabe *et al*., 1999). Also, RAPD based fingerprinting was used successfully in wheat to assess genetic diversity (He *et al.***,**1992, Dhaliwal *et al.*,1993; Cao *et al*.,1999 Kudriavtsev *et al*.,2003; Munshi *et al*.,2003 ; Maric *et al.*, 2004 and Abd-El-Haleem *et al.*, 2009). The aim of this work were to: (1) characterize three durum and one bread Egyptian wheat cultivars at the DNA level using RAPD markers and at protein level using SDS-PAGE and (2) determine the genetic relationships among these genotypes.

2. Materials and Methods

Germplasm material

In the present study three Egyptian durum wheat (*Triticum turgidum* L.) cultivars (Beni Suif 4, Beni Suif 5, Beni Suif 6) and one bread wheat (*T. aestivum*) cultivar (Sids 12) provided by Wheat Research Dept. of the ARC, Egypt were used.

SDS-PAGE

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to study the banding patterns of the studied genotypes, Protein fractionation was performed on vertical slab (16.5 cm x 18.5 cm x 0.2 cm) Hoefer E600, Amersham Pharmacia biotech. According to the method of Laemmli (1970) as modified by Studier (1973).

Extraction and purification of genomic DNA

A modified CTAB (hexadecyl trimethyl ammonium bromide) procedure based on the protocol of Porebski *et al*. (1997) was adopted for obtaining good quality total genomic DNA.

Half gram of fresh leaves from each genotype were collected from one week old seedlings

germinated from seeds of each genotype and quickly frozen in liquid nitrogen and then ground using mortar and pestle. Five ml of CTAB extraction buffer (60ºC), 50 mg PVP (polyvinyl pyrolidone) and 15 µl β-mercaptoethanol (0.3%) were added to leaf powder. The tubes were mixed by inversion and incubated at 65ºC for one hour. Then, 6 ml of chloroform : isoamyl alcohol (24:1) was added and contents were mixed by inversion to form an emulsion. The tubes were centrifuged at 5000 rpm for 20 min at room temperature. The top aqueous layer was further centrifuged at 5000 rpm after addition of 6 ml of chloroform: isoamyl (24:1). Half-volume of 5 M NaCl and two volumes of cold isopropanol were added to the supernatant and mixed well. The tubes were incubated at –20ºC overnight, then centrifuged at 8000 rpm for 15 min. The supernatant was discarded, the pellet washed with 70% cold ethanol, and dried in speed vacuum for 10 min. The pellet was dissolved in 300 µl TE buffer (pH 8.0) overnight at 4-6ºC, then transferred to 1.5 ml epindorfe tube. To remove RNA contamination, 4 µl (10 mg/ml) RNase A (Sigma Co., USA) were added to the DNA solution and incubated at 37ºC for 2 hours. The extracted DNA was deproteinized by adding 4 µl (1mg/ml) proteinase K (Sigma Co., USA) and incubating at 37ºC for 2 hours. Three hundred µl of Tris-saturated phenol-chloroform were added, and mixed by inversion. Tubes were centrifuged at 14000 rpm for 15 min in a microfuge (Eppendorf, USA). The upper layer was transferred to new tubes using wide bore pipette tip and 150 µl of TE buffer was added to the phenol phase, mixed and spun for 10 min. Then the upper layer containing the DNA was removed and added to the sample. DNA was precipitated overnight at –20ºC using 0.1 volume 3 M sodium acetate (pH 8.0) and two volumes of chilled absolute ethanol. The samples were centrifuged at 14000 rpm at 4ºC for 15 min. The DNA was washed with 70 % ethanol, briefly air-dried and re-dissolved in TE buffer.

Estimation of DNA concentration

DNA concentration was determined by diluting the DNA 1:5 in dH_2O. The DNA samples were electrophoresed in 0.7% agarose gel against 10ug of a DNA size marker (Lambda DNA digested with *HindIII* and Phi x 174 DNA digested with *HaeIII*). This marker covers a range of DNA fragments size between 23130bp and 310bp, and a range of concentrations between 95 ng and 11 ng. Thus, estimation of the DNA concentration in a given sample was achieved by comparing the degree of fluorescence of the unknown DNA band with the different bands in the DNA size marker.

RAPD analysis

A set of nine random 10-mer arbitrary primers (Table 1) was used in the detection of polymorphism among the four wheat genotypes. These primers were synthesized on an ABI 392 DNA/RNA synthesizer (Applied Biosystems) at AGERI. RAPD assay was preformed as described by Williams *et al*. (1990) with some modifications. The amplifications reactions were carried out in a volume of 25 µl containing 20ng genomic DNA, 25 pmoles primer, 2mM dNTPs, 2mM $MgCl_2$ and 2 U Taq polymerase (Fermentas) with, 1 x PCR buffer.

Table 1. Sequence of the nine ten-decamer arbitrary primers used in RAPD analysis to detect polymorphism among four wheat genotypes.

No.	Name	Sequence
1	OP-R14	5´ TCCGCTCTGG 3`
2	OP-R17	5´ AGGGAACGAG 3`
3	OP-R20	5´ GGACCCTTAC 3`
4	OP-F11	5´ ACGGATCCTG 3`
5	OP-F14	5´ GGTGATCAGG 3`
6	OP-F15	5´ CCGAATTCCC 3`
7	OP-F16	5´ GGGAATTCGG 3`
8	OP-F18	5´ GGGATATCGG 3`
9	OP-F19	5` CCAAGCTTCC 3`

Thermocyling profile and detection of the PCR products

PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 1 min, an annealing step at 36ºC for 1 min and an elongation step at 72ºC for 1.5 min. The primer

extension segment was extended to 7 min at 72ºC in the final cycle.

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a Polaroid camera. Amplified products were visually examined and the presence or absence of each size class was scored as 1 or 0, respectively.

RAPD data Analysis

The banding patterns generated by RAPD-PCR markers analyses were compared to determine the genetic relatedness of the genotypes. Clear and distinct amplification products were scored as '1' for presence and '0' for absence of bands. Bands of the same mobility were scored as identical.

The genetic similarity coefficient (GS) between two genotypes was estimated according to Dice coefficient (Sneath and Sokal, 1973).

Dice formula: GSij = 2a/(2a+b+c)

Where GSij is the measure of genetic similarity between individuals i and j, a is the number of bands shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i.The similarity matrix was used in the cluster analysis. The cluster analysis was employed to organize the observed data into meaningful structures to develop taxonomies. At the first step, when each accession represents its own cluster, the distances between these accessions are defined by the chosen distance measure (Dice coefficient). However, once several accessions have been linked together, the distance between two clusters is calculated as the average distance between all pairs of accessions in the two different clusters. This method is called unweighted pair group method using arithmetic average (UPGMA) according to (Sneath and Sokal, 1973).

3. Results and Discussion

Genetic diversity based on SDS- PAGE

SDS banding patterns was used to fingerprint three durum and one bread wheat cultivars. Dry wheat seeds were ground into soft flour and water soluble protein fraction was extracted. The total number of bands ranged from eight to seven bands (Figure 1). Sex bands were monomorphic while the others were polymorphic. Beni Suif 4 was characterized by the absence of band -3 with 41.56 kDa. Because of the very low level of a polymorphism a dendrogram for genetic distance and similarity matrix based on SDS-PAGE could not be performed.

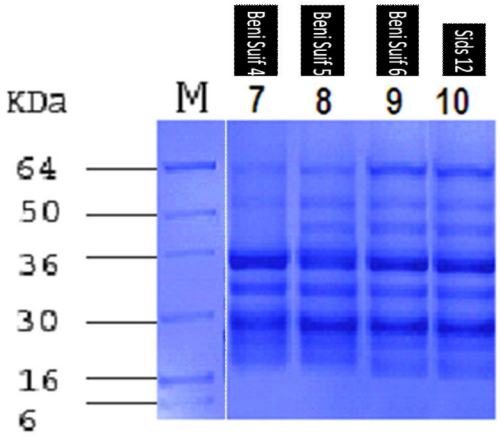

Figure 1. SDS- PAGE for four wheat genotypes

RAPD analysis for genetic diversity among wheat genotypes

Different methods are available for analysis of genetic diversity among germplasm accessions. These methods have relied on morphological, agronomic and biochemical data and recently on DNA-based marker data that allow more reliable differentiation of genotypes. In the present study, twenty ten-mer arbitrary primers were initially screened for PCR amplification of the genomic DNA for the four wheat genotypes. Only nine primers generated reproducible and easily scorable RAPD profiles.

The number of amplified fragments from the genomic DNA of each of the five wheat genotypes generated by the different primers is presented in Table (2). Each of the nine primers produced multiple band profiles with the five wheat genotypes. The highest number of amplicons (14 amplicons) was generated by the primer OPR17 in the genomic DNA of the genotype Beni Suif 4, while the primer OPF15 exhibited the only 2 amplicons, which were monomorphic across the four wheat genotypes.

As shown in Table (3) the total number of DNA fragments amplified by the nine primers was 93 with an average of 10.33 amplicons per primer. The number of polymorphic amplicons ranged from 0 to 15. Primer OPR16 amplified the highest number of polymorphic amplicons, while, the primer OPR14 revealed a total of 6 amplicons which were all monomorphic across the four wheat genotypes.

Table 2. Number of amplified DNA fragments produced by each RAPD primer for the four wheat genotypes.

Primer	Beni .4	Beni.5	Beni.6	Sids12	Total	Mean
OPR14	8	11	12	12	43	10.7
OPR17	14	11	15	12	52	13
OPR20	8	9	9	8	34	8.5
OPF11	6	4	4	8	22	5.5
OPF14	3	3	3	4	13	3.3
OPF15	2	3	3	4	14	3
OPF16	9	4	9	7	29	7.25
OPF18	5	3	4	5	17	4.25
OPF19	3	3	3	3	12	3
Total	58	51	62	63	234	58.6
Mean	6.4	5.7	6.9	7	26	6.5

Therefore, the different primers expressed different levels of polymorphism, ranging from 0.0% with primer OPF19 to 78.9 % with primer OPR16. The total number of polymorphic bands revealed by the nine primers was 56 and the average number of polymorphic fragments/ primer was 6.2. Thus, the average level of polymorphism was 60 %. The size of amplified fragments varied with the different primers, ranging from 250 to 4000 bp (Figures 2 and 3). In this respect, Joshi and Nguyen (1993) investigated the genetic diversity among 15 wheat varieties (*T. aestivum*) using RAPD analysis. Out of 109 amplified DNA fragments, 41 were polymorphic, representing a level of polymorphism of 65%. Perenzin et al. (1997) utilized 87 RAPD primers to assay the genetic diversity among wheat genotypes. They reported that 304 polymorphic bands were generated with an average of 3.49 polymorphic amplicon / primer.

Table 3. Total number of amplicons, number of monomorphic and polymorphic amplicons and percentage of polymorphism, as revealed by RAPD primers

Primer	Total # of amplicons	#of mono amplicons	# of Poly amplicons	Polymorphism (%)
OPR14	15	6	9	60.0
OPR17	19	4	15	78.9
OPR20	15	4	11	73.3
OPF11	11	4	7	63.6
OPF14	6	5	1	16.6
OPF15	5	3	2	40.0
OPF16	12	4	8	66.6
OPF18	6	3	3	50.0
OPF19	4	4	0	0.00
Total	93	37	56	
Average	10.33	4.11	6.2	60.0

Sun et al. (1998) used 32 arbitrary primers for RAPD analysis of 46 wheat genotypes, among which 26 primers (81.3%) revealed polymorphism. A total of 279 amplicons were generated and 182 (65.2%) were polymorphic.

The number of polymorphic amplicons ranged from 2 to 20 with an average of 7 polymorphic amplicons per primer. Zheng et al. (2001) used 55 arbitrary primers in the RAPD analysis of 40 wheat cultivars. Out of 183 amplified fragments, 93 amplicons 50.8% were polymorphic, this represented an average of 1.7 polymorphic amplicons per primer. Moreover, Cao *et al.* (2002) screened 235 random primers against four wheat cultivars to detect RAPD polymorphism. Only, 31 (13.20%) primers produced polymorphism these 31 primers generated a total of 214 reproducible amplified fragments when used with 29 common wheat cultivars. The number of amplified fragments produced by each primer varied from 3 to 12 with an average of 6.9 and an average of 3.10 polymorphic bands per primer. Al-Naggar *et al.* (2004) used 17 arbitrary primers for RAPD analysis of six bread wheat genotypes. Twelve primers (70.60%) generated polymorphic profiles. The total number of amplicons was 98, of which 34 (34.69%) showed polymorphism. Also, Wjhani (2004) studied the genetic variability among 14 wheat accessions using 39 RAPD primers. The total number of amplicons was 117, including 108 polymorphic amplicons. This represented a level of polymorphism of 92.3 % and an average number of 9 polymorphic bands per primer.

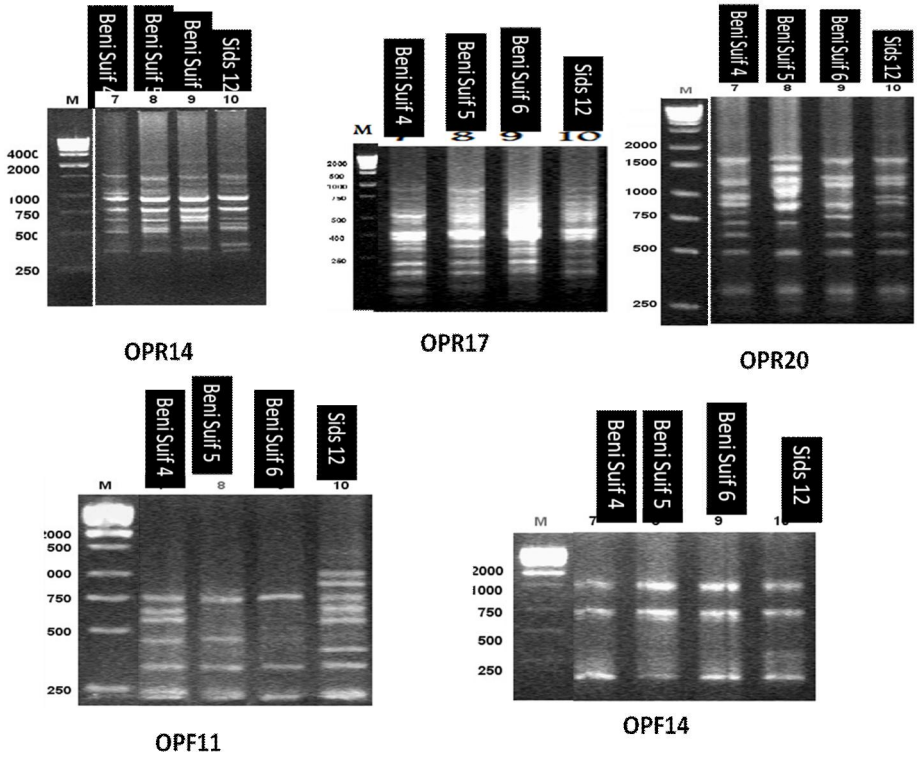

Figure 2. RAPD profile of the three bread wheat genotypes (Beni Suif 4, Beni Suif 5 and Beni Suif 6) and bread wheat genotype Sids 12 amplified with RAPD primers,OPR14,OPR17, OPR20, OPF11and OPF14 : MW : 100 bp ladder.

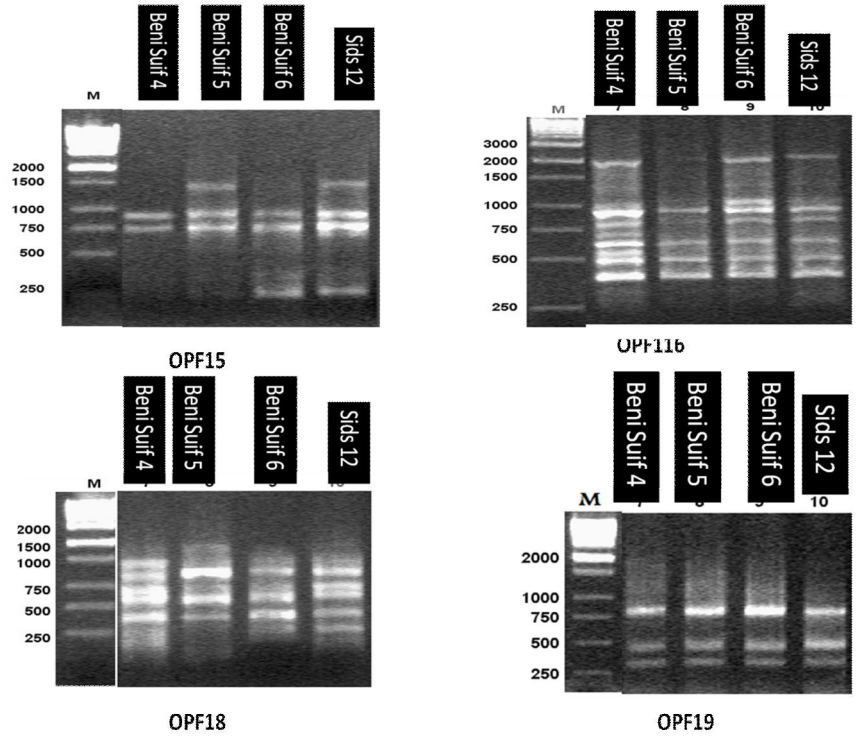

Fig 3. RAPD profile of the three bread wheat genotypes (Beni Suif 4, Beni Suif 5 and Beni Suif 6) and bread wheat genotype Sids 12 amplified with RAPD primers,OPR14,OPR17, OPR20, OPF11and OPF14 : MW : 100 bp ladder.

The results of the present study are in good agreement with those reported in the literature, and confirm that polymorphism is a general phenomenon in in good agreement with those reported in the literature, and confirm that polymorphism is a general phenomenon in wheat although it is a self- fertilizing species.

Genetic relationships among the wheat genotypes

Knowledge of the genetic relationships among genotypes has several important applications in plant breeding programs and plant improvement. It permits the organization of germplasm, including elite lines and provides for more efficient parental selection.

To determine the genetic relationships among the four wheat genotypes, the scoring data (1 for presence and 0 for absence) resulting from the nine tested RAPD primers were used to compute the similarity matrices according to Dice coefficient (Sneath and Sokal 1973). These similarity matrices were then used in the cluster analysis to generate a dendrogram using the UPGMA method. As shown in Table (4) the genetic similarity among the four wheat genotypes ranged from 68.0 to 95.0%.

Table (4). Genetic similarity (GS) matrices among the four wheat genotypes as omputed according to Dice coefficient from RAPDs.

	Beni S.4	Beni S.5	Beni S.6	Sids 12
Beni S.4				
Beni S.5	93.1			
Beni S.6	92.6	95.0		
Sids 12	68.0	65.0	70.0	

The highest genetic similarity revealed by RAPD analysis (95.0%) was between Beni Suif 5 and Beni suif 6 genotypes and followed by (93.1 %) between Beni Suif 4 and Beni suif 6, while the lowest similarity (65 %) was between Beni Suif 4 and Beni Suif 5. This reveals a great concordance between the data deduced from the RAPD analysis and the pedigree of these genotypes.

Cluster analysis as revealed by RAPDs

The Dice RAPD–based coefficients of genetic similarity among the four wheat genotypes were employed to develop a dendrogram using the UPGMA method (Fig.4).The dendrogram separated Sids 12 from all the other genotypes, thus demonstrating the distinctiveness of the genetic background of this genotype from all the other genotypes. The three genotypes constituted a subcluster divided into two groups, one group composed of Beni Suif 5 and Beni Suif 6, while the second group comprised Beni Suif 4. Thus, the dendrogram deduced from the RAPD data corresponded well with the pedigree of the studied wheat genotypes.

The results of the present study revealed, therefore that RAPD analysis is an effective tool for detecting polymorphism, distinguishing between wheat genotypes and assessing their phylogenetic relationships. These results agree with Joshi and Nguyen (1993) who found that analysis of the genetic relationships among wheat varieties could distinguish most of the spring and winter wheat cultivars into different clusters in the dendrogram. Sivolap et al. (1999) reported that RAPD analysis proved to be one of the most powerful methods of discriminating cultivars. The dendrograms based on RAPD markers most closely conform to the pedigree data. Cao et al. (2000) used RAPD marker to assess phylogenetic relationships between 15 wheat accessions. Clusters analysis classified these accessions into five groups in agreement with morphological classification. Sun et al. (2003) found that the dendrogram prepared on the basis of RAPD data corresponded well with the pedigree of two groups of wheat genotypes. Shehata et al (2004) reported that SDS-PAG and RAPD-PCR were successfully used to construct dendrograms to arate the wheat cultivars into two main groups.

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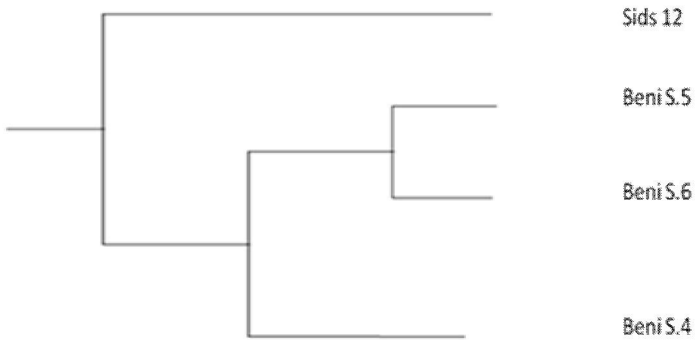

Figure 4. Dendrogram for the four wheat genotypes constructed from RAPD data using (UPGMA) according to Dice coefficients

References

Abd-El-Haleem, S. H. M; Reham, M.A. and Mohamed, S.M. S. (2009). Genetic analysis and RAPD polymorphism in some durum wheat genotypes. Global Journal of Biotechnology and Biochemistry 4 (1): 01-09.

Abdel-Tawab, F. M; Eman M. Fahmy; Bahieldin, A; Asmahan A. Mahmoud; Mahfouz, H.T; Hala F. Eissa and Moseilhy, O. (2003). Marker assisted selection for drought tolerance in Egyptian bread wheat (*Triticum aestivum L.*) Egypt. J. Genet. and Cytol., 32(1): 34-65.

Al-Naggar, A. M.; Sawsan S. Youssef; Ragab, A. F. I and Al-Bakry, M. R (2004). RAPD assessment of new drought tolerance variants derived *via* irradiation and hybridization of some Egyptian wheat cultivars. Egypt. J. Plant Breeding. 8: 255-271.

Cao, W.; Scoles, G.; Hucl, P and Chibbar, R. N. (1999). The use of RAPD analysis to classify *Triticum* accessions. Theor. Appl. Genet., 98: (3-4): 602-607.

Cao, W.; Scoles, G.; Hucl, P. and Chibbar R. N (2000). Phylogenetic
relationships of five morphological groups of hexaploid wheat (*Triticum aestivum* L. em Thell.) based on RAPD analysis. Genome, 43 (4): 724-727.

Cao, W. G.; Hucl, P; Scoles, G; Chibbar, R. N; Fox, P. N; Skovmand, B and Cao, W.G (2002). Cultivar identification and pedigree assessment of common wheat based on RAPD analysis. Wheat Inf. Serv. ,95: 29-35.

Cooke, R. J. (1999). Modern methods for cultivar verification and the transgenic plant challenge. Seed Sci. and Technol., 27:669-680.

Dhaliwal, H. S.; Sidhu, J. S. and Minocha, J. L. (1993). Genetic diversity in diploid and hexploid wheats as revealed by RAPD markers. Crop Improvement., 20, 1:17-20.

El-Khishin. D. A. (1999). Genetic studies in some higher plants using molecular markers. Ph.D. Thesis, Ain Shams Univ., Egypt, 108p

Gupta, P. K.; Varshney, R. K. ; Sharma, P. C. and Ramesh, B. (1999). Molecular markers and their applications in wheat breeding. Plant Breed., 118 (5): 369-390.

Hernandez, P.; Martin, A. and Dorado, G. (1999). Development of SCARs by
direct sequencing of RAPD products: a practical tool for the introgression and marker-assisted selection of wheat. Mol. Breeding, 5: 245-253.

He, S.; Ohm. H. and Mackenzie, S. (1992). Detection of DNA sequence polymorphisms among wheat varieties. Theor. Appl. Genet., 84: 573-578.

Hu, X. Y.; Ohm, H. W. and Dweikat, I. (1997). Identification of RAPD markers linked to the gene PM1 for resistance to powdery mildew in wheat. Theor. Appl. Genet., 94:832-840.

Hussein, Ebtissam, H. A. ; Abd- Alla, S. M.; Awad, Nahla, A. and Hussein , M. S. (2003). Assessment of genetic variability andgenotyping of some Citrus accessions using molecular markers. Arab J. Biotech., 7(1) : 23-36.

Hussein, Ebtissam, H. A. ; Osman, Marwa, H. A.; Hussein, Mona. H. and Adawy, S. S. (2007). Molecular chracterization of cotton genotypes using PCR- based molecular. J. Applied Sci. Res., 3(10): 1156-1169.

Joshi, C. P. and Nguyen, H. T. (1993). RAPD (randomly amplified polymorphic DNA) analysis based intervarietal genetic relationship among hexaploid wheats. Plant Sci. Limerick., 93 (1-2) : 95-103.

Koller, B.; Lehmann, A.; McDermott, J. M. and Gessler, C. (1993). Identification of apple cultivars using RAPD markers. Theor. Appl. Genet., 85:901-904.

Kudriavtsev, A. M.; Martynov, S. P.; M. Brodzhno and Pukhal'skii, V. A. (2003). Evaluation of the relevance of using RAPD-analysis for revealing the phylogenic connections between cultivars of durum wheat (*T. durum* Desf.). Genetika., 39 (9): 1237-1246.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

Malik, T. A.; Pric, A. and Wright, D. (2000). Bulked segregant analysis and RAPD markers for drought resistance in wheat. Pakistan Agric Res., 16 (2): 82-88.

Manabe, M.; Ino, T.; Kasaya, M.; Takumi, S.; Mori, N.; Ohtsuka, I. and Nakamura, C. (1999). Segregation distortion through female gametophytes in interspecific hybrids of tetraploid wheat as revealed by RAPD analysis. Hereditas., Landskrona, 131 (1): 47-53.

Maric, S.; Bolaric, S.; Martinic, J.; Pejic, I.; and Kozumplik, V. (2004). Genetic diversity of hexaploid wheat cultivars estimated by RAPD markers, morphological traits and coefficients of parentage. Plant Breeding., 123(4): 366-369.

Mehboob-ur-Rahman,T; Malik, . A.; Chowdhary, M. A.; Iqbal, M. J. and Zafar, Y. (2004). Application of random amplified polymorphic DNA (RAPD)technique for the identification of markers linked to salinity tolerance in wheat (*Triticum aestivum* L.). Pak. J. Botany., 36 (3): 595-602.

Munshi, A.; Bazaid, S. and El-Tarras, A. (2003). Genetic diversity in diploid KSA wheat as revealed by RAPD analysis. Egypt. J. Genet. Cytol., 32 (1): 25-32.

Nachit, M. M; Monneveux, P.; Araus, J, L.; Sorrells, M. E. ; Royo, M. P. ; Nachit, C, M. M.; Fonzo, N. and Araus, J. L. (2000). Relationship of dry-land productivity and drought tolerance with some molecular markers for possible MAS in durum (*Triticum turgidum* L. var. durum). Durum wheat improvement in the Mediterranean region : New challenges. Proceedings of a Seminar, Zaragoza, Spain., 40 : 203 – 206.

Naqvi, N. I.; Bonman, M.; Mackill, D.; Nelson, R. and Chattoo, B. (1995). Identification of RAPD markers linked to a major blast resistance gene in rice. Mol. Breed., 1:341-348.

Perenzin, M.; M. Corbellini; M. Accerbi; P. Vaccino; B. Borghi; H. J. Braun (ed.); F. Altay (ed.); W. E. Kronstad (ed.); S.P. S. Beniwal (ed.) and A. McNab (1997). Bread wheat: F_1 hybrid performance and parental diversity estimates using molecular markers. Wheat Prospects for global improvement. Proceedings of the 5th International Wheat Conference, Ankara, Turky, 10-14 June 1996. 1997, 339-345; Developments in plant Breeding Volume 6:26.

Porebski, S., Bailey, L. G. and Baum, R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol. Biol. Reporter., 15(1): 8-15.

Rahman, M.; Malik, T. A.; Iqbal, M. J.; Zafar, Y.; Alam, K.; Perveen, Z. and Rehman, S. (1998). DNA marker for salinity resistance. Rice Biotechnology Quarterly., 35 : 12 – 13.

Shehata, M. M. (2004). The roles of seed proteins and RAPD-PCR in genotyping variabilities of some wheat (Triticum vulgareL.) cultivars. Pakistan Journal of Biological Sciences, 7(6):984-994.

Sivolap, Y. M.; Chebotar, S. V.; Topchieva, E. A.; Korzun, V. N. and Otskiy, V. N. (1999). RAPD and SSR analyses of molecular-genetic polymorphism in *Triticum aestivum* L. cultivars. Genetika Moskva, 12: 1665-1673.

Sneath, P.H.A. and Sokal, R.R. (1973). Numerical Taxonomy. Freeman, San Francisco, California, 513 p.

Studier, F. W. (1973). Analysis of bacteiophage T7 early RNAs and proteins of slab gels. Journal of Molecular Biology, 79: 237 -24.

Sun, G.; Bond, M.; Nass, H.; Martin, R. and Dong, Z. (2003). RAPD polymorphisms in spring wheat cultivars and lines with different levels of *Fusarium* resistance. Theor. Appl. Genet., 106:1059-1067.

Sun, Q.; Zhongfu, N.; Zhiuong, L.; Jianwei, G. and Huang, T. (1998). Genetic relationships and diversity among Tibetan wheat, common wheat and European spelt wheat revealed by RAPD markers. Euphytica., 99: 205-211.

Tao, Y., J.M. Manners, M.M.Ludlow and R.G. Henzel (1993). DNA polymorphisms in grain sorghum (*Sorghum biocolor* L. Moeench). Theor. Appl.Genet., 86 : 679-688.

Teshale, E. T.; Bansal, S.; Mishra, A.; Khanna, V. K.;
Bansal, S. and Mishra, A. (2003). DNA fingerprinting of wheat genotypes by RAPD markers. Wheat Inf. Serv., 96: 23-27.

Welsh, J. and McCleland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucl. Acids Res., 18: 7213-7218.

Williams, J. G. K; Kubelik, A. R.; Livak, K. J.; Rafalski, J. A. and Tingey, S.V.(1990). DNA polymorphisms amplified by arabitrary primers are useful as genetic markers. Nucl. Acids Res., 18: 6531-6535.

Wjhani, Y. M. (2004). Genetic Studies on the Biodiversity of Local and Wild Syrian
Wheat Using Modern Biotechnological techniques. PH.D.

Zheng, Y.L.; Chen, Y.Q.; Wei, Y. M.; Zhou, Y. H.; Zhang, Z. Q.; Liu, D. C; Lan, X. J.; Yan, Z. H.; Zheng, Y. L.; Chen, Y. Q.; Wei, Y. M.; Zhou, Y. H.; Zhang, Z. Q.; Liu, D. C.; Lan, X. J. and Yan, Z. H. (2001). Esterase, gliadins and RAPD variations among Sichuan wheat cultivars. Wheat Inf. Serv., 92: 5-8.Thesis, Fac. Agric.,Cairo Univ., Egypt, 175 p.

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