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RAPD ASSESSMENT OF NEW DROUGHT TOLERANT VARIANTS DERIVED *VIA* IRRADIATION AND HYBRIDIZATION OF SOME EGYPTIAN WHEAT CULTIVARS

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ABSTRACT

RAPD markers were used to characterize the genetic differences among six Egyptian bread wheat genotypes, i.e. three drought tolerant variants (V1, V2 and V3) and three Egyptian cultivars (Sakha 8, Giza 164 and Sids 1). Out of the 17 arbitrary primers used, amplification products of 12 primers (70.60 %) generated polymorphic RAPD profiles. The total number of amplicons produced by the 12 polymorphic RAPD profiles was 98.00 with an average number of 8.16 fragments per primer. The number of amplified DNA amplicons across the 12 primers ranged from 6 to 12 amplicons. The total number of monomorphic amplicons was 65.00 with an average number of 5.41 amplicons per primer. The number of amplified monomorphic DNA amplicons across the 12 primers ranged from 3 to 10 amplicons. The total number of polymorphic amplicons was 34.00 with an average number of 2.83 amplicons per primer. The number of amplified polymorphic DNA amplicons across the 12 primers ranged from 1 to 7 amplicons. A maximum number of 12 amplicons was amplified with the primer OPB-15, while, a minimum number of 6 amplicons was amplified with the primers OPB-06 and OPB-16. The highest number of polymorphic amplicons (7) was produced by the primer OPB-15. The percentage of polymorphism ranged from 10.00% shown by the primer OPA-11 to 62.50% revealed by the primer OPA-12. The highest genetic similarity (96.0%) was found between Sids 1 and the waxy mutant (variant 1) and the lowest (86.4%) was found between V3 and its maternal parent (Sakha 8). Two positive and 8 negative unique RAPD markers were identified across the 6 wheat genotypes. OPB-04 primer was able to identify the parent Giza 164 by one positive unique marker at the molecular weight of 557.557 bp. The waxy mutant (V1) was identified by one positive unique marker amplified by OPB-15 primer at the molecular weight of 580.388 bp.

Key words: *RAPD analysis, Polymorphism, Unique marker, Genetic similarity, Waxy (glaucous) mutant, Drought tolerant variant, Wheat, Triticum aestivum.*

INTRODUCTION

Elite breeding lines selected from segregating generations following hybridization and/or mutagen treatments needed to be genetically assessed in order to assure their divergence from their origins and to protect breeder's right. Traditionally, the assessment of the genetic variation in crop plants has been conducted on the basis of phenotypic and cytogenetic characters, which frequently lack the resolving power needed to identify individual genotypes (Teshale *et al* 2003). Estimation from biochemical markers viz isozyme analysis also may be biased by the general consideration that 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 etc. 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 (Miller and Tanksley 1990 and Gupta *et al* 1999). Among the various techniques, RAPD analysis is a potentially simple, rapid, reliable and effective method for detecting polymorphism in wheat (Vierling and Nguyen 1992, Sun *et al* 1998, Gupta *et al* 1999, Cao *et al* 1999 , 2002 and Teshale *et al* 2003).

One of the most important features of the RAPD technique is detecting high levels of polymorphism, an important feature for genetic differentiation. This feature of RAPD has been met in many studies (Sun *et al* 1998, Cao *et al* 2002 , El-Shenhab 2003). This technique has been extensively used for varietal identification, phylogenetic relationships and parentage determination in a wide range of plant species including wheat because of its simplicity and accuracy (Sun *et al* 1998, Gupta *et al* 1999 and Cao *et al* 1999 , 2002). The objectives of this study are to (1) study the polymorphism among wheat new selected variants and their original parents *via* RAPD analysis, (2) assess the genetic relationships among 6 wheat genotypes and (3) identify the genotypes by unique RAPD markers.

MATERIALS AND METHODS

Three new wheat *Triticum aestivum* variants namely: a glaucous (waxy) mutant (V1) selected from the irradiated Egyptian cultivar Sids1, in the M1 generation, and two drought tolerant variants (V2 and V3). The V2 was selected from the irradiated cross Sids 1 X Giza 164, in the F_2M_2 generation and the V3 was selected from the non-irradiated cross Sakha 8 X Giza 164, in the F_2 generation. Their parents Sakha 8, Giza 164 and Sids 1,

were used for RAPD analysis to assure their genetic variability from the original parents on the molecular level.

Isolation of DNA from wheat tissue

Total DNA was isolated according to the method of Sun *et al* (1996). Samples of young leaf tissues were lyophilized overnight, and ground to fine powder using mortar and pestle. 5 ml of prewarmed (60 °C) CTAB extraction buffer (containing 110 mM Tris-HCl, pH 8.7, 55 mM EDTA, pH 8.0, 1.54 M NaCl, 1% CTAB) and 30 µl 1% proteinase K were added, votexed briefly, then 660 µl 20% SDS were added and mixed briefly by inverting. Tubes were placed into a water bath set at 65 °C for 2h (mixed for every 20-min). The tubes were removed from the water bath and allowed to cool down to approximately 30 °C, 5-ml chloroform/isoamyl alcohol (24/1) were added, the tubes were shaken horizontally for 20 min low speed, and centrifuged for 20 min at 5000 rpm. The supernatant was removed into clean tubes, and DNA was precipitated with 2/3 volume of isopropanol. Precipitated DNA was hooked up with a glass rod, washed with 70% precooled ethanol, and dissolved in double distilled water. DNA was diluted in sterile water to a working concentration of 20 ng/µl.

Polymerase chain reaction and electrophoresis

The 17 arbitrary primers used in this study (Table 1) are 10-mer oligonucleotides. These arbitrary primers were synthesized on an ABI 392 DNA/RNA synthesizer (Applied Biosystems) at the Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Egypt. Polymerase chain reaction was performed with a Perkin-Elmer/DNA Thermal Cycler 2400 (Norwalk,CT) in a 50 µl reaction volume, where the lower volume (7 µl) contained 0.5 µl of 10X PCR buffer (0.5 M HCl, 100 mM Tris-HCl, pH 9.0 and 1% Triton X-100), 1.5 µl of 25 mM MgCl2, 2.5 µl solution of each dATP, dCTP, dGTP and dTTP, and 2.5 µl of primer (10 ng / µl). A wax bead was added, the tube was heated to 78 °C for 12 min and then cooled. The upper volume (18µl) contained 2 µl of 10X PCR buffer, 1.5 µl template DNA (20 ng/µl), 0.5 µl Taq Polymerase (2.5 units/µl) and 14.5 µl distilled water. Following an initial denaturation of 3 min at 94 °C, the PCR was carried out for 45 cycles. The cycle program consisted of a denaturation step of 1 min at 94 °C, an annealing step of 2 min at 36 °C, and an extension step of 2 min at 72 °C. The DNA marker, 1Kb was used. This DNA marker covers a range of DNA fragments size between 12216 bp and 201 bp.

Table 1. Nucleotide sequences of the seventeen oligonucleotide primers used in this study.

Serial No.	Primers	Sequences 5′ - 3′
1	OPB-03	5′ CAT CCC CCT G 3′
2	OPB-06	5′ TGC TCT GCC C 3′
3	OPB-12	5′ CCT TGA CGC A 3′
4	OPA-10	5′ GTG ATC GCA G 3′
5	OPB-04	5′ GGA CTG GAG T 3′
6	OPB-07	5′ GGT GAC GCA G 3′
7	OPB-10	5′ CTG CTG GGA C 3′
8	OPB-11	5′ GTA GAC CCG T 3′
9	OPB-13	5′ TTC CCC CGC T 3′
10	OPB-16	5′ TTT GCC CGG A 3′
11	OPB-17	5′ AGG GAA CGA G 3′
12	OPB-19	5′ ACC CCC GAA G 3′
13	OPB-15	5′ GGA GGG TGT T 3′
14	OPB-20	5′ GGA CCC TTA C 3′
15	OPA-11	5′ CAA TCG CCG T 3′
16	OPA-18	5′ AGG TGA CCG T 3′
17	OPA-12	5′ TCG GCG ATA G 3′

DNA data analysis

Data were scored on the presence or absence of amplification products. If a band was present in a genotype it was designated as 1, if no shared band was present in another genotype they were designated as 0 . The data matrix was then used to generate genetic similarity index (GS) according to Nei and Li (1979) as follows: $GS_{(ij)} = 2N_{ij}/(N_i + N_j)$

Where, $GS_{(ij)}$ is the genetic similarity between i and j individuals, (N_{ij}) is the number of bands shared by i and j, (N_i) is the number of bands present in i, and (N_j) is the number of bands present in j. A phenogram was constructed based on genetic distance (GD = 1-GS) using the unweighted pair-group method with arithmetical average (UPGMA).

RESULTS AND DISCUSSION

Polymorphism detected by RAPD markers

In the present study, 17 arbitrary primers were used for PCR amplification of the genomic DNAs of the six bread wheat genotypes under study (Fig. 1, 2, 3, 4 and 5). Among the 17 arbitrary primers, amplification products of 12 primers (70.60 %) generated polymorphic RAPD profiles.

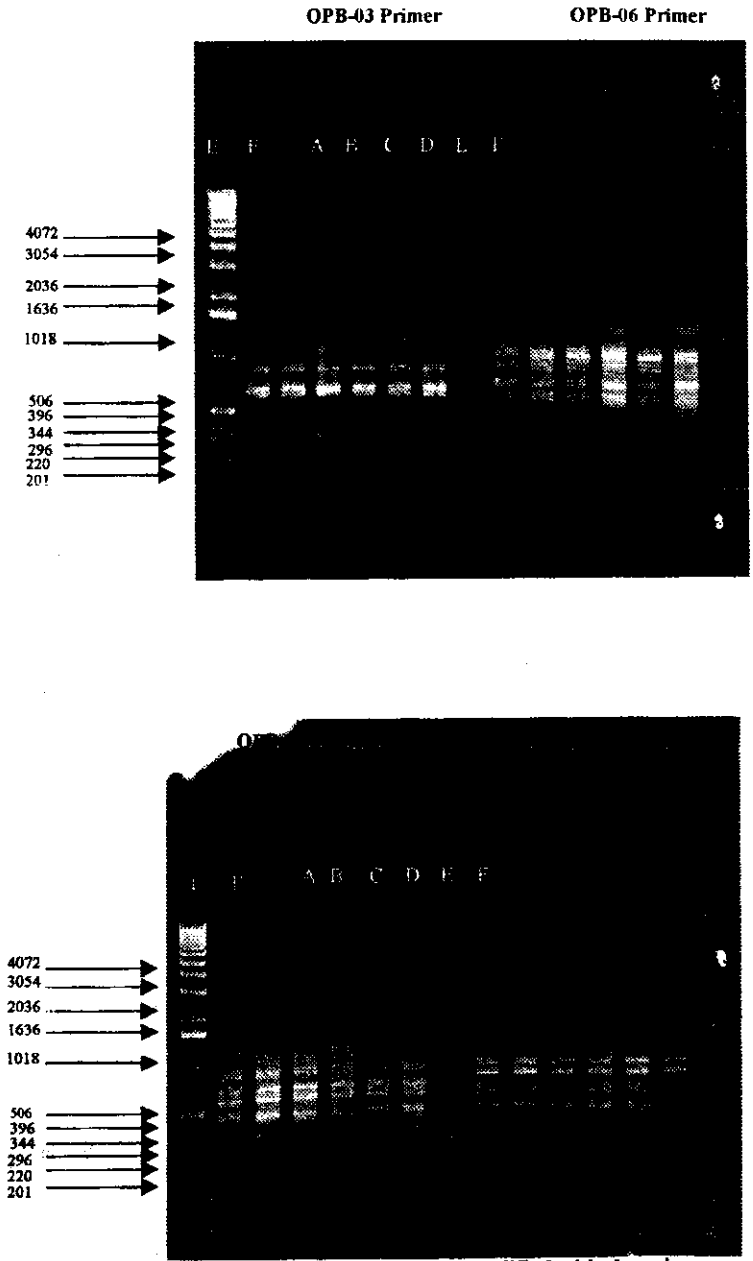

Fig 1. RAPD banding patterns amplified with the primers OPB-03, OPB-06, OPB-12 and OPA-10 for Sakha 8 (A), Giza 164 (B),Sids 1 (C), variants, V1 (D), V2 (E) and V3 (F). M: DNA Marker (1 Kb DNA ladder).

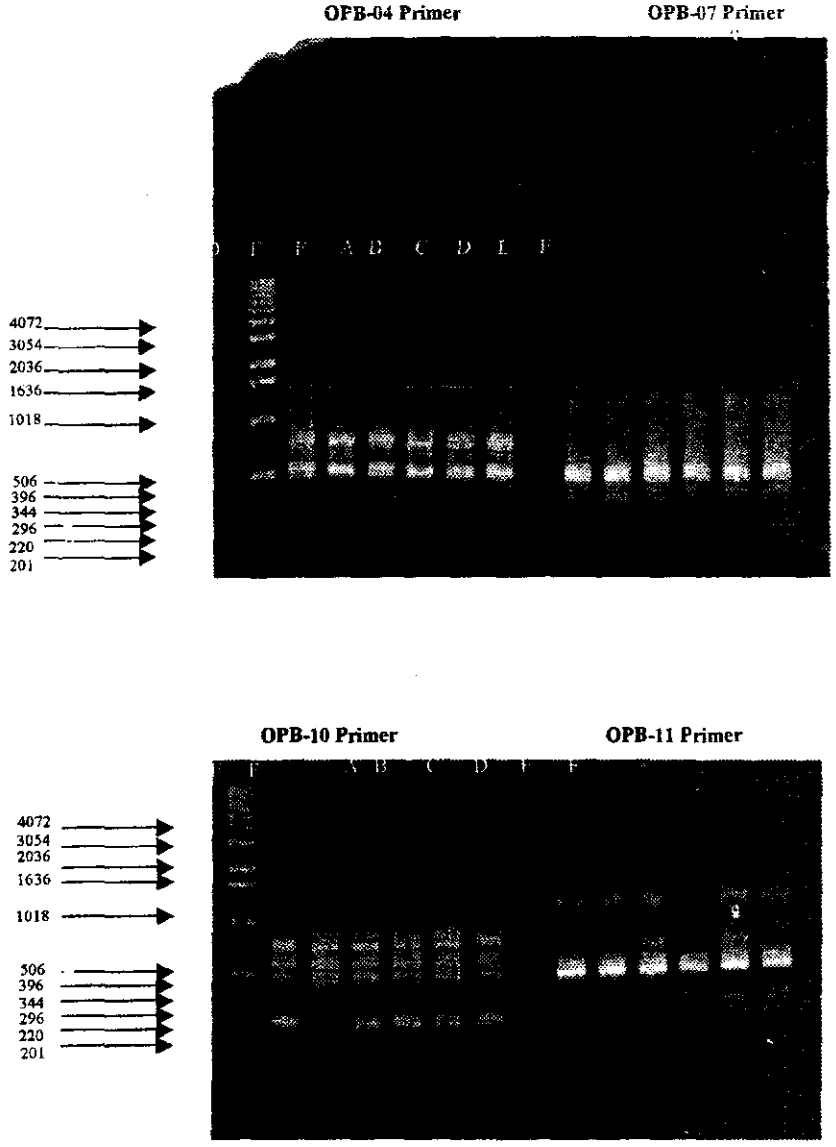

Fig 2. RAPD banding patterns amplified with the primers OPB-04, OPB-07, OPB-10 and OPB-11 for Sakha 8 (A), Giza 164 (B), Sids 1 (C), variants, V1 (D), V2 (E) and V3 (F).
M: DNA Marker (1 Kb DNA ladder).

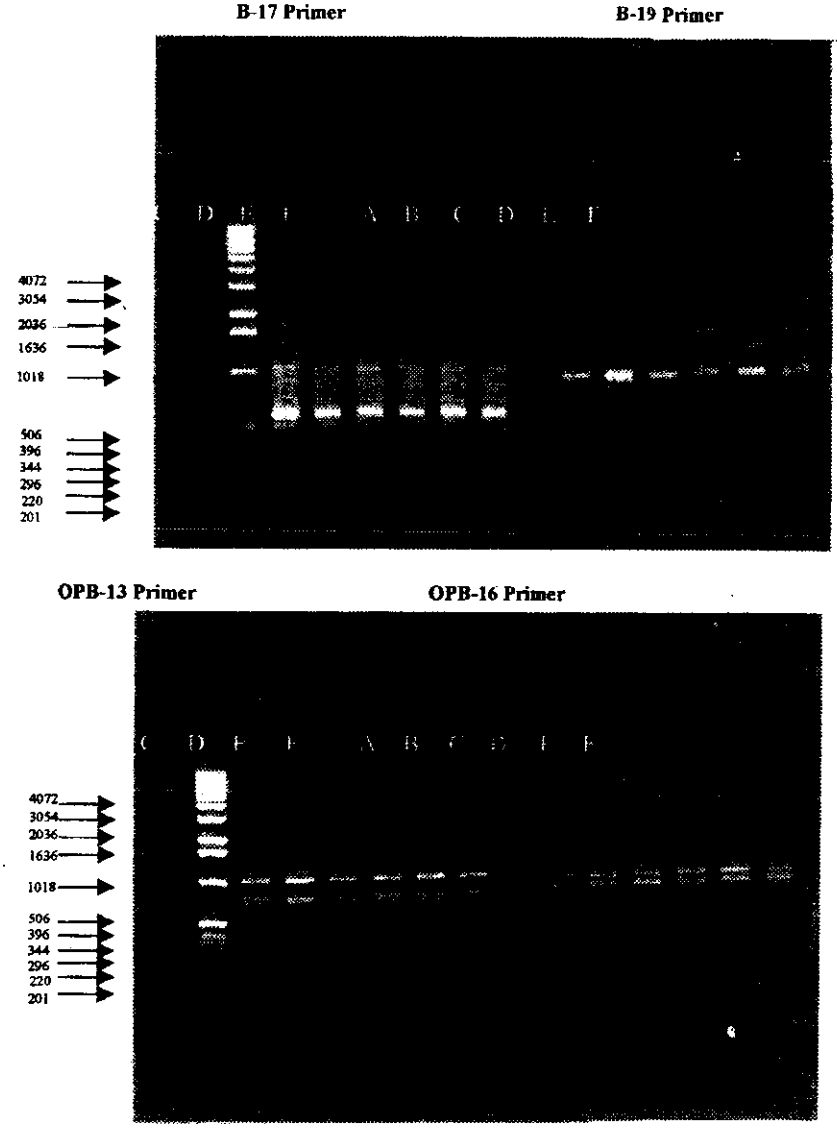

Fig. 3. RAPD banding patterns amplified with the primers B-17, B-19, OPB-13 and OPB-16 for Sakha 8 (A), Giza 164 (B), Sids 1 (C), variants, V1 (D), V2 (E) and V3 (F).

M: DNA Marker (1 Kb DNA ladder).

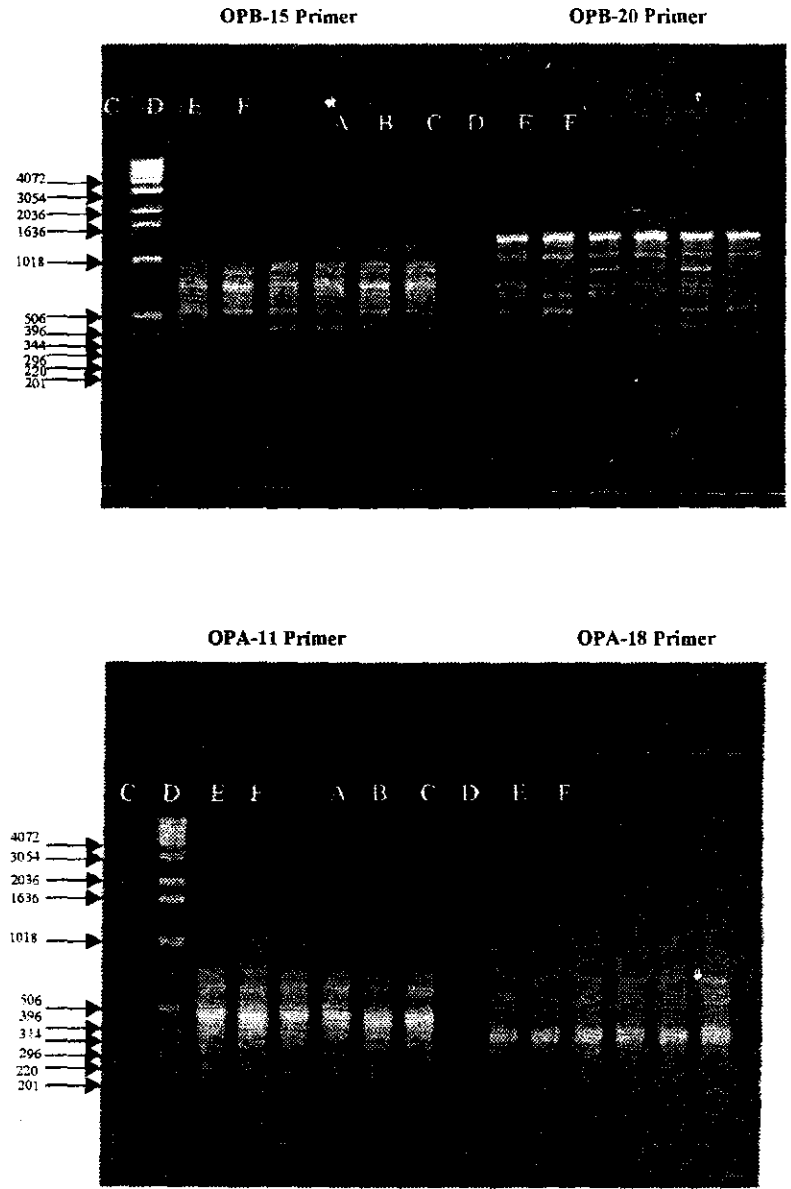

Fig. 4. RAPD banding patterns amplified with the primers OPB-15, OPB-20, OPA-11 and OPA-18 for Sakha 8 (A), Giza 164 (B), Sids 1 (C), variants, V1 (D), V2 (E) and V3 (F). M: DNA Marker (1 Kb DNA ladder).

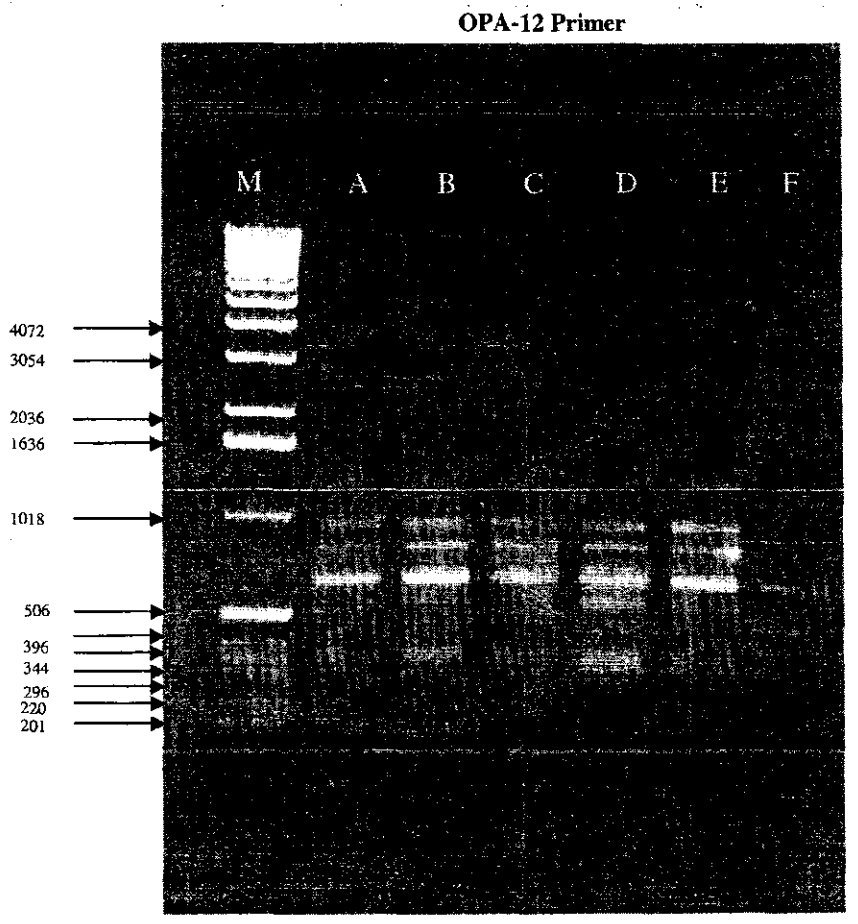

Fig. 5. RAPD banding patterns amplified with the primer OPA-12 for Sakha 8 (A), Giza 164 (B), Sids 1 (C), variants, V1 (D), V2 (E) and V3 (F). M: DNA Marker (1 Kb DNA ladder).

The total number of the amplified RAPD bands produced by the 17 arbitrary primers is presented in Table (2). The number of such amplified bands produced per primer varied from 24 produced by B-19 primer to 54 amplified bands generated by OPB-15 primer.

Table 2. Number of amplified DNA bands scored for the six wheat genotypes.

Primers	No of amplified DNA bands						Total	Mean
	Sakha 8	Giza 164	Sids 1	Variant 1	Variant 2	Variant 3		
OPB-06	5	6	5	6	4	6	32	5.33
OPB-03	6	6	6	6	6	6	36	6.00
OPB-20	6	6	8	8	7	8	43	7.17
OPB-15	8	8	8	10	10	10	54	9.00
OPB-16	5	6	6	6	6	5	34	5.67
B-19	4	4	4	4	4	4	24	4.00
OPA-18	8	8	9	9	9	9	52	8.66
B-17	7	7	7	7	7	7	42	7.00
OPA-10	7	7	7	7	7	7	41	7.00
OPB-12	9	8	8	8	7	7	47	7.83
OPA-11	8	8	8	8	8	8	48	8.00
OPB-13	4	4	4	4	4	4	24	4.00
OPB-10	6	7	7	7	7	6	40	6.67
OPA-12	8	8	8	8	8	3	43	7.17
OPB-11	7	7	7	7	7	7	42	7.00
OPB-04	6	7	7	7	7	6	40	6.67
OPB-07	5	5	6	6	6	6	34	5.67
Total	109	112	115	118	114	109	676	112.83
Mean	6.40	6.88	6.76	6.94	6.70	6.41	39.76	6.63

The total number of amplicons produced by the 12 polymorphic RAPD profiles was 98.00 with an average number of 8.16 fragments per primer (Table 3). The number of amplified DNA amplicons across the 12 primers ranged from 6 to 12 amplicons (Table 3). The total number of monomorphic amplicons (the same bands that can be amplified from one individual to the next according to Hartl and Jones 2001) was 65.00 with an average number of 5.41 amplicons per primer. The number of amplified monomorphic DNA amplicons across the 12 primers ranged from 3 to 10 amplicons. However, the total number of polymorphic amplicons (those bands that can be amplified from some genomic DNA samples but not from others, this means that the presence or absence of the amplified fragment is polymorphic in the population of organisms according to Hartl and Jones, 2001) was 34.00 with an average number of 2.83 amplicons per primer. The number of amplified polymorphic DNA amplicons across the 12 primers ranged from 1 to 7 amplicons. A maximum number of 12 amplicons was amplified with the primer OPB-15, while, a minimum number of 6 amplicons was amplified with the primers OPB-06 and OPB-16. The highest number of polymorphic amplicons (7) was produced by the primer OPB-15. However, the percentage of polymorphism ranged from 10.00% shown by the primer OPA-11 to 62.50% revealed by the primer OPA-12.

Table 3. Total number of amplicons, monomorphic and polymorphic amplicons and percentage of polymorphism as revealed by RAPD markers among wheat genotypes.

Primer	# of amplicons	# of monomorphic amplicons	#of polymorphic amplicons	%of polymorphism
OPB-06	6	4	2	33.33
OPB-03	8	5	3	37.50
OPB-20	9	5	4	44.44
OPB-15	12	5	7	58.33
OPB-16	6	4	2	33.33
OPB-11	8	6	2	25.00
OPA-11	10	9	1	10.00
OPB-12	9	7	2	22.22
OPB-10	7	6	1	14.28
OPA-12	8	3	5	62.50
OPB-04	8	6	2	25.00
OPB-07	7	4	3	42.86
Total	98.00	65.00	34	408.79
Average	8.16	5.41	2.83	34.06

In bread wheat, several studies conducted using RAPDs also found variation among primers in % of polymorphism. Myburg *et al* (1997) fingerprinted five South African cultivars and five Russian cultivars of bread wheat and one cultivar each of triticale and rye using 29 RAPD primers. Their study resulted in the identification of cultivar-specific, genome-specific and species-specific markers through RAPD analysis. Perenzin *et al* (1997) when assayed some wheat parents for RAPD with 87 primers, they reported that 304 polymorphic bands were generated and the genetic similarity between parents, estimated on the basis of common bands, ranged from 0.25 to 0.57. Sun *et al* (1998) used 32 arbitrary primers for PCR amplification of the total DNAs of 46 wheat genotypes, among which amplification products of 26 primers (81.3%) showed polymorphism. A total of 279 products were amplified against the 46 genotypes, among which 182 products (65.2%) were polymorphic. Two to 20 polymorphic products were amplified by each primer yielding 7 polymorphic products per primer on average. Zheng *et al* (2001) used 55 arbitrary primers in the RAPD analysis and generated 183 bands in 40 wheat cultivars, 93 bands (50.8%) of them were polymorphic with an average of 1.7 polymorphic bands per primer. Cao *et al* (2002) screened 235 random primers (10 and 9-mer) against four wheat cultivars to detect RAPD polymorphism. They found that one 132 (56.20%) primers produced fragments that were monomorphic across the four cultivars, 31 (13.20%) primers produced polymorphism.

When they used these 31 primers against another 29 common wheat cultivars, a total of 214 reproducible amplified fragments were generated. They reported that the number of fragments produced by each primer varied from 3 to 12 with an average of 6.9 per primer and the size of fragments ranged from 280 bp to 2800 bp. They found that out of the 214 amplified fragments, 54.70% were monomorphic and 45.30% were polymorphic, with an average of 3.10 polymorphic bands per primer. They detected 97 polymorphisms among the 29 wheat cultivars with the 31 pre-selected random 9- or 10-base primers.

Our results assure the conclusion reported by many other workers that RAPD polymorphism among wheat cultivars are sufficient to allow some of them to be distinguished.

On the other hand, Devos and Gale (1992) detected only a few polymorphism in hexaploid wheat, attributing this to the large portion of repetitive DNA in the common wheat genome. However, their conclusion was based on data from only six primers.

Genetic relationships among the 6 studied wheat genotypes

In the present study, the scored data (1 for presence and 0 for absence) from the 17 primers were used to compute the similarity matrices. The genetic similarity matrices based on the RAPD-based genetic distance were used in the cluster analysis to generate the dendogram using UPGMA analysis. The genetic similarity ranged from 86.4% to 96.0% (Table 4). The highest genetic similarity (96.0%) was found between Sids 1 and the waxy mutant (variant 1) selected from irradiated Sids 1 with 30 Krad of gamma rays which is logic. The genetic similarity between the two parents Sakha 8 and Giza 164 was 91.7%. The genetic similarity between the variant 3 (V3) and its original paternal parent Giza 164 (91.0%) was relatively higher than between V3 and its maternal parent Sakha 8 (86.4%). From these results, it could be concluded that variant 3 combined more genes from Giza 164 than from Sakha 8 and this new combination resulted as a transgressive segregant which was found superior to both of its parents.

Many investigators found wide variation in genetic similarity among wheat genotypes using RAPD analysis .Zheng *et al* (2001) found that the RAPD-based genetic similarity among Sichuan wheat cultivars ranged from 80.4 to 99.1%, with an average of 88.7.%

Table 4. RAPD-based genetic similarity (GS) matrices of the 6 wheat genotypes computed based on the RAPD-based genetic distance.

	Sakha 8	Giza 164	Sids 1	Variant 1	Variant 2
Giza 164	91.7				
Sids 1	91.8	92.8			
Variant 1	91.5	91.6	96.0		
Variant 2	91.5	90.7	93.4	92.2	
Variant 3	86.4	91.0	88.4	89.0	90.7

Cluster analysis

In order to study the genetic relationships among the studied wheat parents and the selected drought tolerant variants derived from them, the matrix of genetic distance was used for cluster analysis by using UPGMA method, and a phenogram was constructed (Figure 6). It showed that the wheat genotypes were distinctly separated into 2 groups. Group 1 included the drought tolerant variant selected from Sakha 8 X Giza 164 (variant 3) and its parents Giza 164 and Sakha 8. Group 1 could also be separated into 2 subgroups, in the first subgroup the two parents Sakha 8 and Giza 164 were closely related. The second subgroup included the transgressive segregant (V3), which showed in the figure closer relationship to Giza 164 than Sakha 8. Group 2 included Sids 1, derived variant from it (V1) and its progenator V2. The parent Sids 1 showed more close relationship to its derived waxy mutant (V1) than the drought tolerant variant 2 (V2). The later variant exhibited closer relationship with Sids 1 than with Giza 164. Also, group 2 could be separated into 2 subgroups, the first one included Sids 1 and V1 and the second included V2.

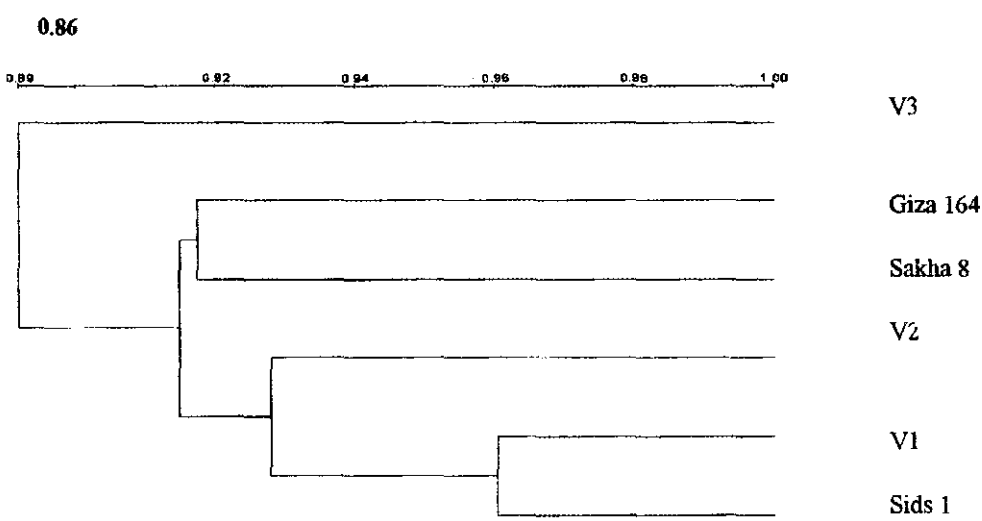

Fig. 6. Dendrogram for the 6 wheat genotypes from RAPDs data using unweighted pair group arithmetic average (UPGMA) and similarity matrices computed according to genetic distance.

Unique markers are defined as bands that specifically identify an accession from the others by their presence or absence. The bands present in one accession but not found in the others are termed positive unique markers (PUM) in contrast with the negative unique markers (NUM), which are absent in a specific genotype. These bands could be used for genotype identification (El-Shenhab, 2003).

Unique positive and/or negative markers and their molecular weight (size) generated by RAPD analysis of the 6 wheat genotypes tested in the current study are presented in Table (5). Two positive and 8 negative unique RAPD markers were identified across the 6 wheat genotypes. One positive unique marker identified the parent Giza 164 generated by the OPB-04 primer at the molecular weight of 557.557 bp. One positive unique markers amplified by OPB-15 primer at the molecular weights 580.388 bp identified the waxy mutant (V1). One negative unique marker amplified by OPB-06 primer at the molecular weight of 1396.70 bp characterized the drought tolerant variant (V2). One negative unique marker generated by OPB-16 with sizes of 912.212 bp characterized Sakha 8. The drought tolerant variant (V3) showed the highest number of negative unique markers (6) generated by OPB-16 and OPA-12 primers at the molecular weights 848.506, 1337.473, 1003.845, 856.601, 582.745 and 259.675. Each of the primers, OPB-15 and OPB-04 generated one positive unique marker. On the other hand, the primers OPB-06, OPB-16 and OPA-12 generated 1, 2, and 5 negative unique markers, respectively. The remaining 11 primers were considered neither unique positive nor negative markers.

Table 5. Positive and negative unique RAPD markers and their molecular weight generated for the 6 wheat genotypes under study.

Genotype	Positive unique marker			Negative unique marker			Grand total
	Size	Primer	Total No	Size	Primer	Total No	
Sakha 8	----	----	----	912.212	OPB-10	1	1
Giza 164	557.557	OPB-04	1	----	----	----	1
V1	580.388	OPB-15	1	----	----	----	1
V2	----	----		1396.705	OPB-06	1	1
V3	----	----	----	848.506 1337.473 1003.845 856.601 582.745 259.675	OPB-16 OPA-12	1 5	6
Total			2			8	10

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تقييم الرابيد لمنتخبات جديدة متحملة للجفاف ناتجة بواسطة الإشعاع والتهجين لبعض أصناف القمح المصرية

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قسم المحاصيل١- قسم الوراثة٢-كلية الزراعة-جامعة القاهره

وحدةالتقنية الحيوية النباتية- قسم البحوث النباتية-مركز البحوث الزراعية-هيئة الطاقة الذريه٣

أستخدمت معلمات الرابيد RAPD لتوصيف الإختلافات الوراثيه بين ستة تراكيب وراثيه من قمح الخبز المصرى وهى ثلاثة منتخبات متحمله للجفاف (الطفرة الشمعية V1 المنتخبة من سدس١ المشعع و والمنتخب الثانى V2 من الهجين سدس ١ X جيزه ١٦٤ و المنتخب الثالث V3 من الهجين سخا ٨ X جيزه ١٦٤ وآبائها الثلاثة (سخا ٨ وجيزه ١٦٤ وسدس ١). من بين ١٧ بادئ عشوائى استخدمت فى هذه الدراسة للتقييم الوراثى (على المستوى الجزيئى) أعطى ١٢ منها اختلافات على مستوى ال DNA . كان العدد الكلى لشظايا (باندات) لDNA الناتج من ال ١٢ بادئ هو ٩٨ شظية (باندا) بمتوسط ٨,١٦ شظية لكل بادئ. وتراوح عدد شظايا الـ DNA عبر الإثنى عشر بادئ بين ٦ و ١٢ شظية وكان العدد الكلى لشظايا ال د ن أ وحيدة الشكل (مونومورفيك) ٦٥ بمتوسط قدره ٥,٤١ شظية لكل بادئ. بينما كان العدد الكلى لشظايا ال DNA متعددة الشكل (بولى مورفيك) ٣٤ بمتوسط قدره ٢,٨٣ شظية لكل بادئ. أعطى البادئ OPB-15 أكبر عدد من الشظايا وهو ١٢ شظية، بينما أقل عدد من الشظايا وهو ٦ نتج من البادئين OPB-16 و OPB-06. تراوحت نسبة الاختلافات (البولى مورفيزم) ما بين ١٠% (ناتج البادئ OPA-11) و ٦٢,٥% (ناتج البادئ OPA-12). كان أعلى نسبة تشابه وراثى (٩٦%) بين الأب سدس١ و الطفرة الشمعية V1. وكان التشابه الوراثى بين

الأبوين سخا ٨ وجيزة ١٦٤ قدره ٩١,٦% وكان بين المنتخب الثالث V3 وكل من أبويه جيزة ١٦٤ وسخا ٨ قدره ٩١% و ٨٦,٤% على التوالى. تميزت الستة تراكيب الوراثية تحت الدراسة إلى مجموعتين. تضمنت المجموعة الأولى المنتخب الثالث V3 وأبويه جيزة ١٦٤ وسخا ٨. تضمنت المجموعة الثانية سدس١ والمنتخب منه V1 والمنتخب الثانى V2. تم تحديد واسمين فريدين موجبين وثمانية واسمات فريده سالبة عبر الستة تراكيب الوراثية تحت الدراسة. تميز كل من الأب جيزة ١٦٤ وكذلك الطفرة الشمعية (V1) بواسم موجب فريد لكل منهما بواسطة البادئين OPB-04 و OPB-15 على التوالى. كما تميز الصنف سخا ٨ عن طريق واسم سالب نتج عن طريق البادئ OPB-16. أظهر المنتخب الثالث V3 المتحمل للجفاف أكبر عدد من الواسمات السالبة الفريدة (٦) نتجت عن طريق البادئين OPB-16 و OPA-12 .