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The Roles of Seed Proteins and RAPD-PCR in Genotyping Variabilities of Some Wheat (*Triticum vulgare* L.) Cultivars

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Abstract: Traditional identification of wheat cultivars relies on the assessment of agronomic traits of the adult plant. This leads to a significant delay of time, constraints to breeders in the surveillance of germplasm and a risk for growers and exporters. Seed storage protein and DNA fingerprinting diversity as revealed by variation in SDS-PAGE and RAPD-PCR, respectively has been used to reassess the genotypic differences, relationships and discriminating between eight wheat (*Triticum vulgare* L.) cultivars (Banisewef 3, Gemmiza 7, Giza 164 and 168, Sakha 8, 69 and 93 and Sids 1) grown in Egypt. A total number of 46 protein bands, with molecular weight ranging between 160.52 and 3.86 kDa were recorded in the electrophenograms of the cultivars studied. SDS-PAGE profiles showed slight differences at high and low molecular weights and high differences at mid molecular weights. The highest number of bands (30) was recorded in Banisewef 3 and the lowest number (21) was recorded in Sids 1. Eight common bands (M.wts.; 155.32, 139.50, 76.35, 29.25, 19.25, 8.20, 7.20 and 6.38 kDa) were recorded in all samples. Six unique bands were recorded in cultivars Banisewef 3 (M.wts: 132.69 and 73.50 kDa), Sakha 8 (M.wt.: 38.50 kDa) and Sakha 69 (M.wts.: 88.59, 63.20 and 24.85 kDa). In the RAPD-PCR analysis twenty five random decamer (10 mer) primers were screened, but only eight primers (OPB-01, -02, -03, -08 and OPI-03, -09, -14 and -18) were able to generate distinguishable, reproducible and repeatable informative products among the DNA samples of the studied genotypes. A total of 67 polymorphic bands out of 86 ones were generated by the eight primers. Nearly every cultivar has a different DNA fingerprint. Each cultivar has one or more novel sequences not found in other cultivars. These bands may be used as genetic markers for identification of these cultivars using the same primer. A total of 8 polymorphic bands were scored as unique ones. Primers OPI-09 and -03 were found to be the most effective in generating unique bands. The former primer generated 4 unique bands in Banisewef and Giza 164 cultivars while the latter primer produced 2 unique bands in Giza 169 and Sakha 93 cultivars. Increasing and changing the number of cycles in PCR from 30 to 40, improve our results. These bands were used as binary characters and analyzed by the NTSYS-pc. Program Package using the UPGMA clustering method. This analysis indicates that, the eight cultivars are distinct. SDS-PAGE and RAPD-PCR data were combined together and used to construct a dendrogram that estimates the relationship and the relative genetic similarities among the studied wheat cultivars. Based on this dendrogram, the studied cultivars were separated into two main groups. The first group comprises three cultivars, while the second includes five cultivars. The first group was further subdivided into two subgroups; the first subgroup comprises cultivar Sakha 8, whereas the second subgroup includes cultivars Sakha 69 and Sakha 93. The second group was further subdivided into two subgroups; the first subgroup comprises cultivars Giza 169 and Giza 164, whereas the second subgroup includes cultivars Sids 1, Gemmiza 7 and Banisewef 3. Combination of all data provides a considerable potentiality for discriminating each wheat cultivar by one or more unique bands or a group of combined class pattern. As a result of this investigation, we may expect that the SDS-PAGE, RAPD and the subsequent banding patterns computed using appropriate programs, would be useful for the establishment of phylogenetic relationships among a set of Egyptian wheat cultivars. These tools could be used as complementary of traditional methods of identification of phenotypic traits for the control of registered cultivars in the trade market. This will help in collection and cataloguing of the germplasm in the form of a germplasm bank.

Key words: Wheat cultivars, SDS-PAGE, RAPD-PCR, genotypes similarity index, dendrogram

INTRODUCTION

The world population is increasing at the rate of 96 to 100 million people each year – or nearly the size of another Mexico City every 12 weeks[1]. Grasses provide our food and are economically the most valuable group of organisms on the planet. The Food and Agriculture Organization[2] considered the cultivated wheat as the first most important cereal crop in the world. A side from just a couple of limiting nutrients which can easily be made up with small quantities of other foods, wheat has long been considered the focal point of home food storage. Wheat has been called the "staff of life" for hundreds of years because of it's excellent nutrition, storability and versatility. The genus *Triticum* belongs to the Gramineae family. The chromosome numbers may be 14 (diploid), 28 (tetraploid) or 42 (hexaploid). Wheat is closely related to other cereal grains, especially rye, barley and oats. Wheat grains contain considerable proportion of proteins and other important nutrient elements. Seed storage proteins of cereals consist of several subunits and their expression patterns differ among cultivars[3]. Therefore, they can be used to discriminate among cultivars. Wheat proteins are collectively called "Gluten". Gluten is a mixture of individual proteins classified in tow groups, prolamins and the glutelins.

The different cultivars of wheat grown today probably show little resemblance to wheat grown thousands of years ago. Plant breeders have had hundreds of years to carefully modify this grain to produce quicker in areas of short summers, be more drought resistant and have higher yields. Each cultivar has been enhanced with the positive characteristics for it's intended use. Traditional cultivar identification based on morphological traits requires extensive observations of mature plants[4]. Furthermore, morphological traits cannot serve as unambiguous markers because of environmental influences[5]. Nowadays, biochemical and molecular markers have been developed to solve this problem. Gel electrophoresis of protein has become a standard and powerful research tool for application in biological sciences. The development of cultivar-specific genetic markers is desirable for cultivar identification and protection as well as seed purity determination. Moreover, knowledge of genetic diversity in economical plant gene pools is central to germplasm conservation strategies[6].

Electrophoretic assays have been widely used as a rapid and accurate test to identify and characterize different crop cultivars. By the use of appropriate and refined techniques, it is now possible to actually "fingerprint" each cultivar to assess its identity and its agronomic properties. SDS-PAGE and RAPD-PCR analyses were successfully used for both identification and differentiation of plant cultivars. They reliably rapid means for establishing genetic profiles and elucidation of genetic relationships within and between taxa[3,7-11]. SDS-PAGE was carried out by several investigators for identification and characterization of mutants in maize[12] and ananas[13].

DNA analysis, now a frequent courtroom evidence tool, is being fine-tuned by an Agricultural Research Service scientist as a faster way to develop and fingerprint new rice and wheat varieties to feed a hungry world. The researchers also used the common sequence to isolate others that produce a DNA fingerprint capable of both identifying and cataloging genetic differences within rice and wheat. The development of RAPD-PCR markers by Williams[14] have a number of advantages over the other DNA-based markers systems[15]. RAPD fingerprinting is a polymerase chain reaction (PCR) based technique that yields DNA products ranging in size from 200-2000 bp. When these products are separated on an electrophoretic gel, a banding pattern is observed that is very similar to a marketing bar code used in many stores. RAPD is an effective, simpler and more rapid than other molecular methods such as restriction enzyme analysis or pulsed field gel electrophoresis. It may be used for analysis of many plant samples using small quantities, pedigree analysis[16,17], molecular mapping[18], taxonomy and phylogeny[19], identification of genotypes associated with genes of interest and genetic diversity studies[20,21] on the basis of the discrete profiles produced. Moreover, they are also useful for detecting chromosome mutations, such as inversion, deletion or translocation[22,23]. With respect to the family Leguminous, some recent examples of PCR studies, mainly on phylogenetic aspects and genetic diversity on the genus level, are those on *Glycine*[24] and *Vicia*[25]. The RAPD fingerprint is characteristic of the target DNA and has been used to differentiate between closely related genotypes of wheat species.

Recently, DNA rather than protein has been exploited for species identification due to its stability at high temperatures and its structure being conserved within all tissues of an individual. The discriminating ability of the RAPD method is virtually unlimited as it is always possible to use other random primers. For plant breeders, DNA fingerprinting using RAPD-PCR can be a quick way to select parents with the widest range of genetic variability. The study of genetic diversity of wheat cultivars is important from the theoretical point of view, in order to understand the evolutionary process for these cultivars and practically to evaluate the potential of the genetic resource for future crop improvement.

Traditional identification of wheat cultivars relies on the assessment of agronomic traits of the adult plant. This leads to a significant delay of time, constraints to breeders in the surveillance of germplasm and a risk for growers and exporters. The aim of the present study was to provide polymorphic biochemical and molecular fingerprints for the identification, characterization and examination of the phylogenetic relationships among eight of Egyptian wheat cultivars to be handy for plant breeders and geneticists in their breeding programs by means of electrophoretic patterns of seed storage proteins (SDS-PAGE) and random amplified polymorphic (RAPD) DNA markers.

MATERIALS AND METHODS

Plant material: The grains of eight wheat (*Triticum vulgare* L.) cultivars (Banisewef 3, Gemmiza 7, Giza 164 and 168, Sakha 8, 69 and 93 and Sids 1) were obtained from the Agricultural Research Center, Ministry of Agriculture, Giza, Cairo, Egypt. The grains were washed several times in tap water and finally with distilled water.

Protein extraction and SDS-PAGE: Ten randomly chosen dry grains of each cultivar were milled separately to fine powder. Protein fractions were extracted using buffer solution (1 M Tris-Cl, pH 6.8, 0.25 M EDTA and 10% SDS). Samples were centrifuged at 14000 rmp for 10 min in a cooling centrifuge at 4°C. The supernatants were used for further analysis. Protein fractions were characterized and identified using one-dimensional SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) technique. Slab gels (12.5%) were prepared according to Laemmli[26]. The destained gel was analyzed by GDS (Model UVP's, GDS 8000, UVP Inc., California 91786, USA) to evaluate the percentage (band%), molecular weight {M.wt. in kDa (kilodalton)} and the relative mobility rate (R_m) of different protein bands in the presence of protein molecular weight marker.

DNA extraction, amplification and agarose gel electrophoresis: Grains of each cultivar were germinated in the greenhouse to mid-tillering stage (20 cm long). Leaves of ten plants were randomly chosen from each cultivar and were harvested. Genomic DNA's were extracted using the 2X CTAB method. DNA was quantitated and qualitated by spectrophotometry and agarose gel electrophoresis.

Polymerase chain reaction amplifications were performed using a PJ2000 Thermal Cycler (Perkin Elmer, Norwalk, Connecticuts, USA) with standard optimized control. Each PCR reaction was repeated three times to ensure repeatability of the amplification patterns. DNA was amplified according to Williams *et al.*[14] using twenty five arbitrary primers (Operon Tech., Kits B and I, USA). RAPD-PCR conditions were optimized for annealing temperatures (32-37°C), numbers of thermal cycles (25-40), DNA template concentration (5-200 ηg) and magnesium chloride concentrations (1-3 mM). The optimal conditions for RAPD-PCR were as follows: PCR was carried out in a volume of 50 µl containing 1x PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 8.0, 0.1% gelatin), 5 ηg DNA, 5 pmol primer, 200 µM dNTPs mix, 1. 5 mM $MgCl_2$ and 0.5 U *Taq* polymerase. The thermal cycling conditions were as follows: 5 min at 94°C (one cycle, hot start), 35 cycles (30 sec at 94°C, 30 sec at 36°C and 60 sec at 72°C) and a final one cycle post-extension of 10 min at 72°C.

The amplified PCR products were electrophoresed on an agarose (1.8 %) (DNA grade, Sigma) in 1x TAE buffer (40 mM Tris-base, pH 8.0, 20 mM glacial acetic acid and 2 mM Na_2-EDTA). Electrophoresis was performed for 1 h at 100 V in submarine electrophoresis cell (Pharmacia). Gels were stained with ethidium bromide for visualization of bands, examined by Gel Doc 2000™ Gel Documentation System (Bio Rad) and video printing of gel image. The RAPD-gels were scanned and analyzed using Gel Pro-Analyzer as in protein analysis. The size of the amplified products was estimated from the gel by comparison to a standard DNA molecular size marker.

Data scoring and statistical analysis of markers: Scoring of bands was made for each cultivar and each primer that yielded a clear pattern. Protein patterns and amplification products were scored as present (1) or absent (0). Pair wise comparison of all the genotypes considered in each survey were used to estimate the genetic distance according to Nei and Li[27] $D=1- (2N_{xy} / N_x + N_y)$, where N_x and N_y are the number of bands present in cultivars x and y, respectively and N_{xy} is the number of bands shared by both cultivars. The dendrogram was constructed by the NTSYS-pc. Program Package using the UPGMA Clustering method.

RESULTS AND DISCUSSION

SDS-PAGE: The most widely used techniques for analyzing mixture of protein is SDS-PAGE. Proteins are separated essentially on the basis of their size. The electrophoretic patterns (SDS-PAGE) for water soluble proteins (albumin and globulin) of eight wheat cultivars are illustrated in Fig. 1 and Table 1. From the SDS-PAGE analysis of water soluble proteins, bands were recorded with different intensities (band%) molecular weights (M.wts) and relative mobilities (R_m). A total number of 46

Table 1: Comparative analysis of relative concentrations (band %), molecular weight (M. wt.) and mobility rate (R_m) of protein bands of eight wheat cultivars separated using SDS-PAGE technique

Lane	Band%									
Band No.	1	2	3	4	5	6	7	8	R_m	M.wt. (kDa)
1	-	0.58	-	-	-	0.38	-	-	0.05	160.52
2	1.30	1.82	-	-	2.35	3.50	4.10	-	0.05	158.40
3	2.35	6.23	1.02	0.69	0.35	2.90	1.23	3.80	0.06	155.32
4	3.20	-	5.20	3.80	9.50	1.02	-	-	0.06	151.62
5	0.36	1.80	1.78	-	2.30	0.98	1.20	3.60	0.06	149.65
6	-	3.52	-	2.20	5.20	-	-	-	0.06	146.25
7	0.68	0.80	2.30	-	-	2.80	-	-	0.07	141.69
8	1.32	0.98	5.20	2.03	0.88	0.56	0.63	4.20	0.07	139.50
9	-	2.30	2.50	1.20	3.20	-	5.20	-	0.08	138.60
10	1.23	-	2.50	-	-	-	3.60	-	0.08	136.37
11	-	-	-	5.20	1.25	-	-	-	0.08	132.69
12	5.20	-	-	-	-	-	-	-	0.09	129.50
13	3.50	-	8.50	0.98	-	-	-	3.20	0.09	118.50
14	-	-	-	2.36	-	-	-	-	0.10	111.63
15	-	5.20	3.28	4.20	1.20	-	-	-	0.11	105.36
16	-	-	-	-	-	-	-	2.58	0.13	98.36
17	4.20	-	-	5.32	-	-	7.50	-	0.14	92.50
18	-	-	-	-	-	2.50	-	-	0.16	88.59
19	9.30	-	-	-	5.20	-	-	3.28	0.17	82.50
20	4.20	1.36	8.20	0.96	0.58	2.36	1.36	7.56	0.19	76.35
21	4.50	-	-	-	-	-	-	-	0.21	73.50
22	1.23	0.85	-	0.69	-	-	-	-	0.24	69.20
23	-	-	-	-	-	6.25	-	-	0.26	63.20
24	0.36	4.25	10.32	-	2.50	1.20	3.20	5.21	0.28	58.25
25	-	1.32	8.50	9.32	5.20	-	1.23	8.25	0.29	52.34
26	3.24	2.35	-	-	-	-	2.69	-	0.31	46.25
27	-	-	-	-	7.38	-	-	-	0.32	38.50
28	-	-	6.35	1.28	-	4.36	-	-	0.36	33.27
29	2.85	-	5.36	8.52	3.20	-	-	-	0.38	31.20
30	1.23	3.33	5.23	0.99	0.85	4.11	1.65	1.03	0.41	29.25
31	2.32	0.85	0.88	3.11	11.20	2.88	0.89	1.55	0.45	26.25
32	-	-	-	-	-	2.66	-	-	0.46	24.85
33	5.20	1.36	4.20	2.22	5.20	2.35	1.25	4.22	0.50	22.38
34	3.55	2.55	4.22	3.58	2.58	3.66	5.86	8.11	0.53	19.25
35	-	2.30	6.33	-	8.56	-	-	5.22	0.58	16.27
36	4.22	-	3.88	9.33	5.23	3.22	3.66	6.88	0.61	14.28
37	-	-	5.22	2.33	6.30	-	3.55	-	0.63	12.50
38	2.58	7.26	3.66	5.26	4.26	6.33	6.33	-	0.67	11.28
39	5.66	-	2.40	1.25	2.33	3.33	6.38	3.66	0.70	9.50
40	4.22	2.55	1.11	1.58	1.36	2.96	1.25	1.28	0.74	8.20
41	2.96	2.35	1.88	5.36	5.12	1.39	10.20	6.34	0.78	7.20
42	4.56	3.90	2.36	5.63	2.35	8.20	2.36	4.66	0.82	6.38
43	2.58	-	-	2.35	-	-	-	-	0.89	5.90
44	4.25	5.28	-	3.20	4.65	6.20	7.10	5.23	0.91	4.53
45	-	-	-	-	-	4.20	5.89	-	0.96	4.10
46	2.36	-	3.20	2.50	-	-	6.20	4.50	0.97	3.86
Total	30	24	27	29	28	25	25	21		

Lane 1: Banisewef 3, lane 2: Gemmiza 7, lane 3: Giza 164, lane 4: Giza 169, lane 5: Sakha 8, lane 6: Sakha 69, lane 7: Sakha 93 and lane 8: Sids 1

Table 2: The codes, sequences and GC% of the used primers and the number of polymorphic and monomorphic bands generated using these primers

Primer	Sequence	GC%	No. of polymorphic bands	No. of monomorphic bands
OPB-01	GTTTCGCTCC	60	5	3
OPB-02	TGATCCCTGG	60	7	6
OPB-03	CATCCCCCTG	70	17	2
OPB-08	GTCCACACGG	70	7	2
OPI-03	CAGAAGCCCA	60	6	1
OPI-09	TGGAGAGCAG	60	15	2
OPI-14	TGACGCGGTG	70	4	-
OPI-18	TGCCCAGCCT	70	6	3
	Total No. of bands		67	19

protein bands, with molecular weight ranging between 160.52 and 3.86 kDa were recorded in the electrophenograms of the cultivars studied. SDS-PAGE profiles showed slight differences at high and low molecular weights and high differences at mid molecular weights. The highest number of bands (30) was recorded in Banisewef 3 and the lowest number (21) was recorded in Sods 1. Eight common bands (M.wts.: 155.32, 139.50, 76.35, 29.25, 19.25, 8.20, 7.20 and 6.38 kDa) were recorded in all samples. Six unique bands were recorded in cultivars

Table 3a and b: RAPD-PCR fragments and their molecular size in base pairs (bp) generated by eight decamer primers (OPB: Table 3a and OPI: Table 3b) in eight wheat cultivars

	Primer															
	OPB-01								OPB-02							
Molecular size (bp)	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
920	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
900	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
800	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
750	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
700	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
680	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
650	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+
630	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-
610	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
600	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-
530	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
500	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+
480	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
450	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
410	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
400	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
350	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
330	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-
320	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+
300	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+
280	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	+
260	+	+	+	+	+	+	+	+	-	-	+	+	+	-	-	+
250	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
230	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
210	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
200	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+
180	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
150	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	+
120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	7	7	6	3	8	8	5	4	8	10	12	13	10	7	6	13
					8								13			

	OPB-03								OPB-08							
Molecular size (bp)	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
920	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
900	+	+	+	+	-	+	-	-	+	+	+	+	-	-	-	-
800	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
750	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+
700	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+
680	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
650	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
630	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
610	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-
600	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-
530	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-
500	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	-
480	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-
450	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
410	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
400	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
350	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
330	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
320	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
300	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
280	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
260	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
250	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
230	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
210	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-
200	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
180	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
150	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
120	-	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-
Total	11	15	15	14	10	14	6	5	9	8	6	6	5	3	3	2
					19								9			

Lane 1: Banisewef 3, lane 2: Gemmiza 7, lane 3: Giza 164, lane 4: Giza 169, lane 5: Sakha 8, lane 6: Sakha 69, lane 7: Sakha 93 and lane 8: Sids 1.

Table 3b: Continue

	Primer															
	OPI-03								OPI-09							
Molecular size (bp)	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
930	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
900	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
850	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
810	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
800	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
700	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
680	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
660	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
640	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-
620	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
580	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
500	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
470	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
430	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
400	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
380	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-
360	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
350	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
330	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
320	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
310	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
300	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
280	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-
260	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
250	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-
220	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
200	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+
180	-	+	+	-	+	+	-	+	-	-	+	-	-	-	-	-
160	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
150	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
130	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Total	2	4	5	5	3 7	2	2	2	4	7	13	7	8 17	8	5	5

	Primer															
	OPI-14								OPI-18							
Molecular size (bp)	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
930	-	-	-	-	-	-	-	-	+	+	+	+	-	+	-	+
900	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
850	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
810	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+
800	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
700	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
680	+	+	+	-	+	-	+	+	-	-	-	-	-	-	-	-
660	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
640	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
620	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-
580	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
500	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
470	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
430	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
400	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
380	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
360	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
350	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
330	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	+
320	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
310	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
300	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-
280	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
260	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-
250	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3b: Continue

Molecular size (bp)	Primer															
	OPI-14								OPI-18							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
220	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
180	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
160	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
150	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
130	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	3	3	2	2 4	1	1	4	3	8	8	9	7	6 9	7	3	6

Lane 1: Banisewef 3, lane 2: Gemmiza 7, lane 3: Giza 164, lane 4: Giza 169, lane 5: Sakha 8, lane 6: Sakha 69, lane 7: Sakha 93 and lane 8: Sids 1.

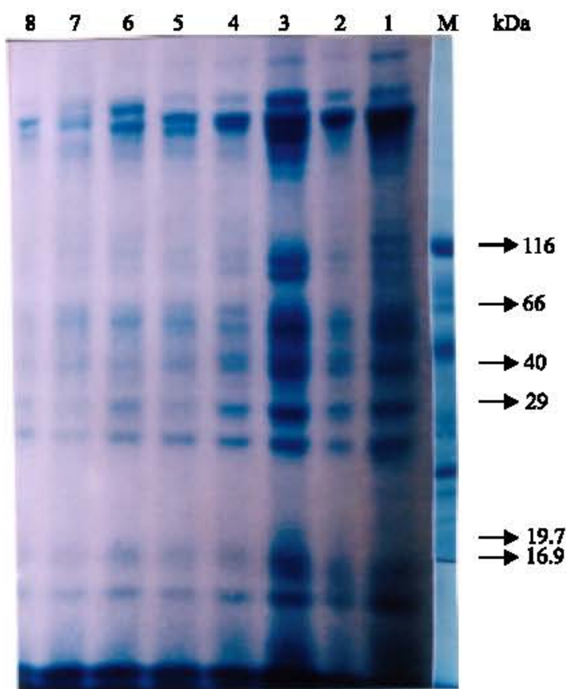

Fig. 1: SDS-PAGE banding patterns of eight wheat cultivars (lanes: 1 - 8). Lane 1: Banisewef 3, lane 2: Gemmiza 7, lane 3: Giza 164, lane 4: Giza 168, lane 5: Sakha 8, lane 6: Sakha 69, lane 7: Sakha 93 and lane 8: Sids 1. Lane M: standard protein molecular weight marker

Table 4: Similarity indices among the eight wheat cultivars as estimated using SDS-PAGE and RAPD-PCR analyses

	1	2	3	4	5	6	7	8
1	100.0							
2	82.0	100.0						
3	64.3	66.6	100.0					
4	62.5	79.2	78.4	100.0				
5	44.7	89.4	82.5	74.4	100.0			
6	85.8	74.6	81.6	73.6	46.2	100.0		
7	79.6	87.3	48.8	77.4	68.3	71.2	100.0	
8	88.2	69.4	58.6	78.9	44.2	68.5	78.4	100.0

1: Banisewef 3, 2: Gemmiza 7, 3: Giza 164, 4: Giza 169, 5: Sakha 8, 6: Sakha 69, 7:Sakha 93 and 8: Sids 1.

Banisewef 3 (M.wts: 132.69 and 73.50 kDa), Sakha 8 (M.wt.: 38.50 kDa) and Sakha 69 (M.wts.: 88.59, 63.20 and 24.85 kDa).

Storage proteins in endosperm of wheat grain represent over 80% of the whole quantity of proteins in grain. These proteins are not soluble in water at neutral pH value. Storage proteins are either soluble in 70% ethanol (gliadins) or insoluble (glutenins). Glutenins have a wide spectrum of molecular weight of 50,000 to several millions Daltons. It is assumed that, the genes which synthesized subunits of glutenin are on the long arms of the chromosomes 1B, 1D and 4D[28].

In general, the electrophoretic separation of water soluble protein indicated the presence of a wide genetic variations among the studied wheat cultivars. These results agreed with those of Chen *et al.*[29]. They found differences in electrophoretic patterns of soluble proteins and suggested that these differences could be used effectively in the identification of wheat cultivars. Our results are also in accordance with those of Perovic *et al.*[30] who used water soluble protein electrophoretic patterns in identifying different barley and wheat genomes A, B and D in addition to the Rye genome (R). Six protein markers were found to distinguish R genome, one marker for cultivars belonging to A genome and three markers for cultivars representing B and D genomes.

The analysis of a large number of wheat cultivars by means of SDS-PAGE has proved that all loci display a large number of allelic variations[31]. He also demonstrated that in hexaploid wheat there are 3 alleles: the allele a controls the synthesis of the subunit 1, the allele b for the synthesis of the subunit 2 and the allele c is not translated into a protein visible on gel. Our results show that protein patterns obtained by SDS-PAGE may be used as a sufficient tool for differentiation of the studied cultivars by comparing the amount and quality of protein bands.

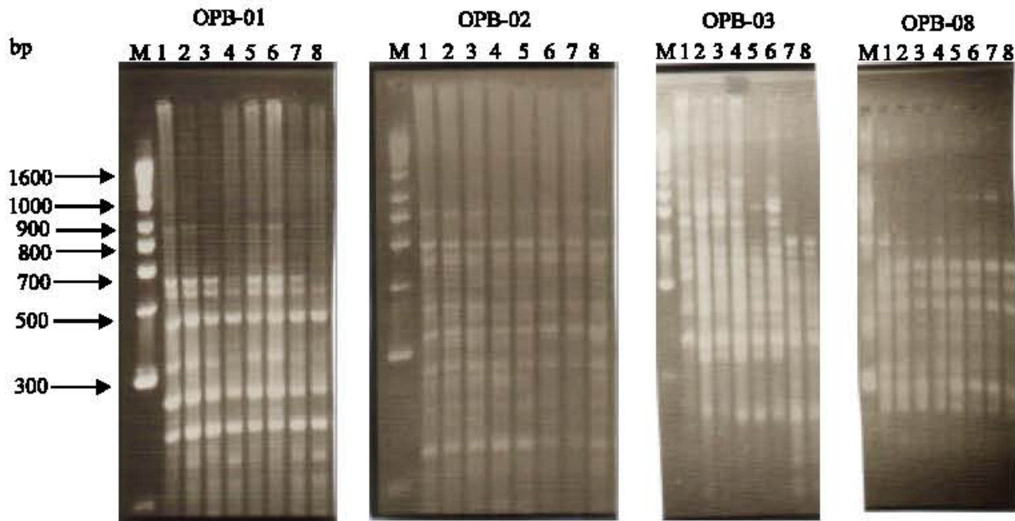

Fig. 2: RAPD fingerprints detected in eight wheat cultivars (lanes: 1- 8) using four decamer primers (OPB-01, -02, -03 and -08). Lane 1: Banisewef 3, lane 2: Gemmiza 7, lane 3: Giza 164, lane 4: Giza 168, lane 5: Sakha 8, lane 6: Sakha 69, lane 7: Sakha 93 and lane 8: Sids 1. Lane M: standard DNA molecular size marker

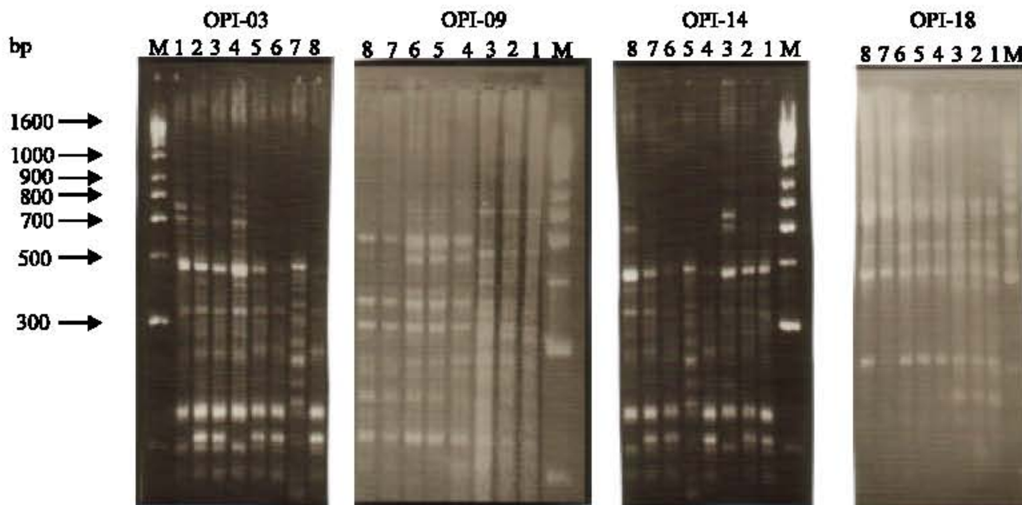

Fig.3: RAPD fingerprints detected in eight wheat cultivars (lanes: 1- 8) using four decamer primers (OPI-03, -09, -14 and -18). Lane 1: Banisewef 3, lane 2: Gemmiza 7, lane 3: Giza 164, lane 4: Giza 168, lane 5: Sakha 8, lane 6: Sakha 69, lane 7: Sakha 93 and lane 8: Sids 1. Lane M: standard DNA molecular size marker

RAPD-PCR: High molecular weight DNA was successfully extracted from all samples. The ratio of A_{260}: A_{280} ranged from 1.6 to 1.84, which was sufficiently pure for PCR reactions. RAPD-PCR reactions were performed with the eight wheat cultivars. Out of the twenty five 10-mer RAPD primers used (Table 2), 8 primers produced distinguishable as well as polymorphic bands in each of the DNA samples of the studied genotypes and seem to be more appropriate for cultivars differentiation. Only one PCR program was used. This was to standardize the evaluation of the primers. The effect of changing PCR conditions on the results was also discussed. The results of amplification for the eight wheat cultivars using eight random primers indicated that, there are distinct differences in the usefulness of different primers for the identification of wheat cultivars (Fig. 2 and 3; Tables 2, 3a and b). They also show the DNA fingerprint of each cultivar, illustrating the high genotype diversity in the

nuclear genome. To demonstrate the utility of these samples of RAPD primers for the detection of genetic variability in wheat cultivars, the number of fragment patterns generated by each primer was recorded for a subset of genotypes comprising the cultivated plants. A total of 67 polymorphic bands out of 86 ones were generated by the eight primers. Nearly every cultivar has a different DNA fingerprint. Each cultivar has one or more novel sequences not found in other cultivars. These bands may be used as genetic markers for identification of these cultivars using the same primer. A total of 8 polymorphic bands were scored as unique ones. Primers OPI-09 and -03 were found to be the most effective in generating unique bands. The former primer generated 4 unique bands in Banisewef and Giza 164 cultivars while the latter primer produced 2 unique bands in Giza 169 and Sakha 93 cultivars (Table 3a and b). The number of fragments amplified using the same primer are not always identical among these cultivars but a few primers shared the same behaviour. The highest number of bands (19) was recorded using primer OPB-03, while the lowest number was obtained using primer OPI-14. Also, the number of total bands varied between cultivars where the lowest numbers is 6 bands in cultivars Sakha 93 and Sids 1 and the highest number is 25 bands in cultivar Giza 164. A band with molecular weight about 500 bp was found to be absent only in cultivar Sids 1, 300 bp in cultivar Sakha 8, 330 and 200 bp in cultivar Sakha 93, which indicated that these bands might be considered as specific marker (negative marker) for this cultivar compared with the others.

Some workers have found that a change of one base pair in the target sequence of the genome may result in a completely different RAPD profile[14]. Since each 10 bp oligonucleotide primer only covers a very limited part of the genome, important differences located on non-amplified regions could be missed. In the event of similar profiles obtained from two different species (especially closely-related species) using a particular primer, this would lead to a false conclusion that the two species are the same. Thus, it is important to use a series of primers for any sample to be tested.

The RAPD analysis of the used primers revealed no specific markers among more than four of the eight wheat cultivars, therefore, it is still effective in cultivar identification. This result was due to low copy DNA sequences produced by these primers. The same conclusion was reached by Perovic *et al.*[30] who detected many low copy DNA sequences among barley genomes which might be parts of euchromation regions for specific functioning genes.

In some cases where no reaction was seen with a particular primer, repeats were carried out. This was to rule out the possibility that the PCR reagents (e.g. *Taq* polymerase, primers) on that a particular day did not work, although this was remote as all the other samples on the same gel used the same PCR cocktail and underwent the same PCR procedure. Generally, experiments carried out with the 70% GC primers showed clear fingerprint patterns, compared to the other primers. These primers could be used to discriminate among the used cultivars.

To analyze the genetic distances within and between the different wheat cultivars, a dendrogram displaying the genetic similarity among the genotypes was constructed. Combination of all data provides a considerable potentiality for discriminating each wheat cultivar by one or more unique bands or a group of combined class pattern. Based on this dendrogram, the studied cultivars were separated into two main groups. The first group comprises three cultivars, while the second one includes five cultivars. The first group was further subdivided into two subgroups; the first subgroup comprises cultivar Sakha 8, whereas the second subgroup includes cultivars Sakha 69 and Sakha 93. The second group was further subdivided into two subgroups; the first subgroup comprises cultivars Giza 169 and Giza 164, whereas the second subgroup includes cultivars Sods 1, Gemmiza 7 and Banisewef 3.

A distance matrix between breeds (Table 4) shows an average distance range from 0.44 to 0.89 with a mean of 0.66. Thus, the breeds tested in this study are highly divergent (mean>0.5) at the DNA level. The smallest distance value was observed between Sakha 8 and Sids 1 cultivars which appear to be the most similar cultivars and can be closely regrouped. The maximum distance value of 0.89, indicating a great dissimilarity, is observed between the Gemmiza 7 and Sakha 8 cultivars. All the other cultivars display different intermediate levels of similarity and are grouped with the other ones. It is noteworthy that the Sakha 8 cultivar presented very limited average distance ranges (from 0.52 to 0.56) with the other ones except with Sods 1, Gemmiza 7 and Banisewef 3. Thus, Sids 1 cultivar could be characterized by a slight divergence at the DNA level and could be unlikely regrouped with the other clusters.

The genetic distance matrix using the UPGMA algorithm was computed to cluster the data and to draw the precise relationships between the eight tested genotypes. The clusters identified significantly supported a breedal branching of only six breeds. These are Sakha 69, Sakha 93, Giza 169, Giza 164, Gemmiza 7 and Banisewef 3 which are characterized by their opposite wheat property. Thus, it has been assumed that the following breeds: Sakha 69/Sakha 93, Giza 169/Giza 164 and Gemmiza 7/Banisewef 3 may constitute paired clusters, while Sakha

8 and Sids 1 may be carefully considered as two single breedal groups with respect to the other clusters. The use of UPGMA cluster analysis for differentiation of plant varieties and taxa was used by some workers such as Wong *et al.*[32] who used eight primers for differentiation between three taxa of *Musa acuminata* cv. Colla. Our results are confirmed by Ko *et al.*[35] who reported that arbitrary 10mer allowed distinction between cultivars of the same species (maize, barley, sorghum, rye, rice, oat and wheat), although some variation was found within cultivars and some primers gave products characteristic of the cereal species. They also postulated that the results might influenced by the primer used in the experiment, quality of the template and alterations in amplification conditions. However, in contrast to his results, increasing and changing the number of cycles in PCR from 30 to 40, improve our results.

When the eight random primers are combined together (Table 3a and b), complete identification was obtained for the studied cultivars. Some of these primers were more successful in cultivar identification such as primers OPB-03 and OPI-09; as they generated a high number of RAPD markers and consequently, more cultivars were classified. These results confirmed that PCR technique is an efficient tool for the identification and characterization of wheat cultivars which agreed with Keller and Feuillet[33] who used PCR technique to distinguish between some wheat species, accessions and cultivars. Similar results were obtained by Sandhu and Gill[34] who used random primers for detecting markers to distinguish wheat and wheat-related genomes. Our results indicates that, RAPDs may be used to quantify genetic variation within and among different cultivars of wheat and the results are considered as an important baseline data for conserving the genetic resources of wheat as the first cereal crop in the world.

Based on few morphological characters, wheat cultivars have been assumed to lack genetic variability and therefore novel strategies to include new germplasm have been suggested to further improve the diversity and quality of new wheat cultivars. Due to the very complex and in large parts unknown or secret breeding history of most present day cultivars, it is almost impossible to estimate cultivars and the genetic diversity within wheat groups on the basis of pedigree data. As a result of this investigation, we may expect that the SDS-PAGE, RAPD and the subsequent banding patterns computed using appropriate programs, would be useful for the establishment of phylogenetic relationships among a set of Egyptian wheat cultivars. It is obviously necessary to enlarge both the number of cultivars and/or the number of primers to obtain a deeper insight of Egyptian wheat cultivars. A prerequisite for achieving this work is the improvement of reliable molecular markers that make possible discrimination between genotypes.

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