RAPD AND SDS-PAGE ANALYSIS OF R_4 SALINITY-TOLERANT WHEAT (*TRITICUM AESTIVUM* L.) REGENERANT LINES

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Abstract

The objective of this study was to examine the banding pattern of four salinity tolerant and four sensitive regenerated (R_4) wheat genotypes. PCR-RAPD Randomly Amplified Polymorphic DNA technique as well as SDS-(PAGE) Sodium dodecyl sulfate polyacrylamide gel electrophoresis were applied on H lines (High-salinity tolerant lines) and L lines (Low-salinity tolerant lines) chosen from R_4 wheat *Triticum aestivum* L. regenerant lines together with their parents (Sakha 8, Lines 25 and 28) under control and salinity stress conditions to determine any associated molecular markers with salinity tolerance/susceptibility genes. Some protein electrophoresis bands disappeared or induced at the two salinity levels in all lines. Among 28 RAPD primers used 16 primers showed reproducible bands and high polymorphism (57.6%). Primer B5 had unique markers to Line 28 (sensitive to salinity) and the four sensitive lines at 900 bp, it's may be fragment sensitive to salinity. A dendrogram separated the lines into two main groups, the highest similarity between 3L and 4L was 92%. The dissimilarity between parent and the regenerant lines shows that somaclonal variation could produce regenerant lines from parent varieties, which are different in their genetic make-up. Molecular markers system showed some markers which are linked to some yield related traits.

Key words: Wheat, *Triticum aestivum*, RAPD, SDS-PAGE, Salinity, Tolerance, Regenerant lines.

INTRODUCTION

Wheat (*Triticum aestivum* L). is one of the main cereal crops in Egypt. The conventional breeding techniques have met limited success in improving the response of many crops to salt stress (Epstein *et al.*, 1980). DNA markers can help to resolve the number of present different genetic classes and genetic similarities among them, and the diversity present in those classes and their evolutionary relationship with wild relatives. Knowledge of genetic relationship among genotypes is useful in plant breeding programs because it permits the organization of germplasm, including elite lines (Adawy *et al.*, 2004). Polyacrylamide gel electrophoresis (PAGE) provided good markers for the identification and characterization of different genotypes. SDS-PAGE is widely used, not only for determination of the protein molecular weight, but also for

the detection of various unknown proteins in addition to the identification and quantitation of newly biosynthesized proteins (Zahur and Robercht, 1979). The accumulation of some new polypeptide bands related to salt treatment and that they could be considered as marker proteins for salt adaptation was shown by some workers (Fahmy *et al.,* 2002). The objective of this study was to examine the banding pattern of four salinity tolerant and sensitive regenerated (R_4) wheat genotypes. PCR-RAPD technique as well as SDS-(PAGE) polyacrylamide gel electrophorasis were applied on H lines (High-salinity tolerant lines) and L lines (Low-salinity tolerant lines) chosen from R_4 wheat (*Triticum aestivum* L) regenerant lines. and to determine any associated molecular markers with salinity tolerance/susceptibility genes.

MATERIALS AND METHODS

SDS-PAGE and RAPD analysis was determined in four salinity tolerant and sensitive regenerated plants (R_4) wheat genotypes (H and L lines). H (high-salinity tolerant lines) and L (low-salinity tolerant lines) lines produced from R_4 wheat *Triticum aestivum* L. regenerant lines (Sabry *et al.*, 2006) together with their parents (Sakha 8, Lines 25 and 28) under control and salinity stress conditions.

A. SDS-PAGE protein electrophoresis:

Sodium dodecyl sulfate polyacrylamid gel electrophoresis (SDS-PAGE) was used to extract water soluble protein from four salinity tolerant and sensitive regenerated R_4 lines (H and L lines) together with their parents (Sakha 8 and Lines 25 and 28) under two salinity levels (0 and 12000 ppm) according to Studier (1973). The samples were randomly chosen from the kernels of each genotype. 0.2g of each kernel sample was ground with 100 ul of water soluble extraction buffer and added at 5°C overnight then centrifuged for 30 min at 14000 rpm at 4°C. The supernatant was used directly or stored at -20° C until use. Electrophoresis was carried out in 12% acrylamide gel using low protein molecular weight marker (Fermentas), 1.5 mm thickness at 4°C until the bromophenol blue front passed completely through the gel. The gel was stained for 12 hrs in 0.1% coomassie brilliant blue and distained until the bands were clear. Gel bands were scanned and analyzed using Gel Doc (Alpha Imager TM 2002).

B. PCR-RAPD technique

PCR (Polymerase Chain Reaction) was used to examine the banding pattern in four H lines and four L lines together with their parents (Sakha 8, and lines 25 and 28).

Genomic DNA was isolated and extracted by CTAB (Hexacetyl Trimethyl Ammonium Bromide).

A total of 28 random 10-mer primers (Table 1) were used in the detection of

polymorphism among the eleven wheat genotypes. RAPD-PCR was carried out according to Williams *et al.* (1990) with minor modifications. Reaction mixtures (25ul) contained 10 X PCR buffer 2.5 ul, 20mM dNTPs 2.5ul, $MgCl_2$ 2ul, 10Pmol primer 2ul, 1unit Taq polymerase 2ul and 10ng DNA extraction, 3ul up to 25 ddH_2O. PCR amplification was performed in a GeneAmp- PCR system 9700 programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94°C. Each cycle consisted of a denaturation step at 94° C for 45 sec, annealing step at 36° C for 1 min and an elongation step at 72° C for 1.30 min. The primer extension segment was extended at 72°C for 5 min in the final cycle. Amplified products were held at 4°C The amplification products were resolved by electrophoresis in a 1.2% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts for two hrs. PCR products were visualized on UV light and photographed. Amplified products were visually examined and the presence or absence of each class was scored as 1 or 0, respectively. DNA molecular weight markers used through out this study was: GeneRuler 50bp DNA Ladder (Fermentas)

Table 1. Catalog number and base sequence of the 28 random primers.

No.	Name	Sequence	No.	Name	Sequence
15	D-10	-5GGTCTACACC	1	Amer Sham 1	-5GGTGCGGGAA
16	D-15	-5CATCCGTGCT	2	Amer Sham 2	-5GTTTGGCTCC
17	D-16	-5AGGGCGTAAG	3	Amer Sham 3	-5GTAGTCCCGT
18	D-19	-5CTGGGGACTT	4	B-1	-5GTTTCGCTCC
19	OPB06	-5CATCCCCCTG	5	B-3	-5CATCCCCCTG
20	OPB010	-5GTGATCGCAG	6	B-5	-5TGCGCCCTTC
21	OPA12	-5TCGGCGATAG	7	B-9	-5TGGGGGACTC
22	tube A11	-5CAATCGCCGT	8	B-10	-5CTGCTGGAAC
23	UBC2	-5CCTGGGCTTG	9	B-13	-5TTCCCCCGCT
24	UBC 61	-5TTCCCCGACC	10	B-17	-5AGGGAACGAG
25	UBC 82	-5GGGCCCGAGG	11	C-20	-5ACTTCGCCAC
26	Z-2	-5CCTACGGGGA	12	D-3	-5GTCGCCGTCA
27	Z06	-5GTGCCGTTCA	13	D-4	-5TCTGGTGAGG
28	Z-12	-5TCAACGGGAC	14	D-5	-5TGAGCGGACA

RESULTS AND DISCUSSION

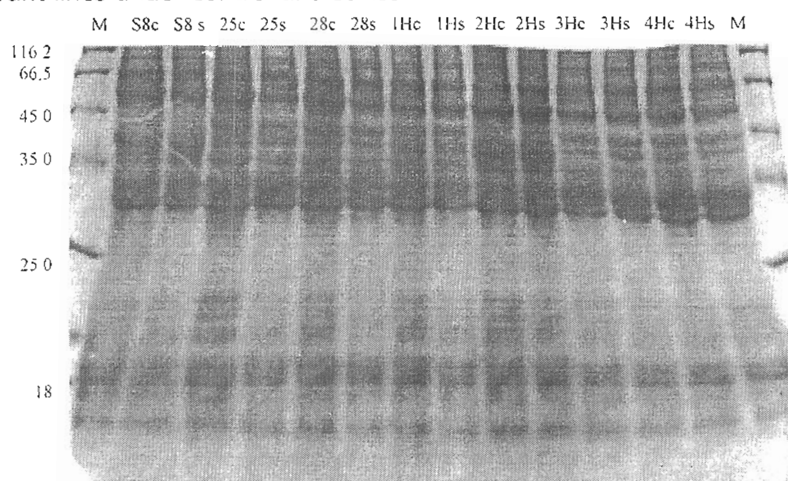
Molecular fingerprints

1. Protein electrophoresis profiles:

SDS-PAGE of soluble protein:

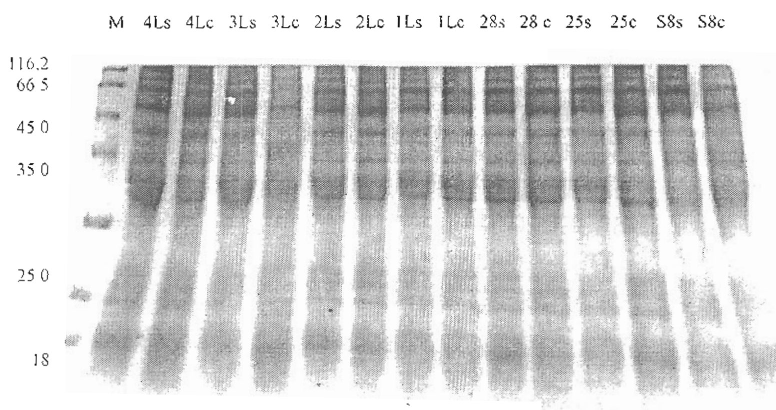
Eleven genotypes under control and salinity stress were fingerprinted by SDS-PAGE of water soluble protein (Figure 1 and 2).

Fig. 1. SDS-PAGE of water soluble protein fraction of three parents and four salinity tolerant lines under control and stress



M= Marker, S=Sakha 8, 25=Line 25, 28=Line 28,
H= high tolerant salinity genotypes, s= salinity stress, c= control

Figure 2. SDS-PAGE of water soluble protein fraction of three parents and four salinity tolerant genotypes under control and stress



M= Marker, L= low tolerant salinity tolerant, S= Sakha 8, 25= Line 25,
28=Line 28, s= salinity Stress, and c= control treatment

A total of 35 bands were detected with different molecular weight ranging from 112.2 to 11.9 kDs (Table 2 and 3). In Table 2, seven genotypes of wheat (Parents Sakha 8, Line 25, and Line 28 with four high tolerant and sensitive salinity lines) under control and high salinity levels was detected. When comparing the protein banding pattern of the genotypes, some additional protein patterns were present while, others were absent in all cultivars. Sakha 8 can be characterized by 23 bands under control and 25 bands under salinity stress. Two bands were found under salinity only (44.17 and 24.0 kD). Line 25 showed 28 bands under control and 26 bands under salinity. It had five bands which appeared under control only (42.51, 34.12, 31.48, 22.25, and 21.50 kD) and four bands under salinity only (44.17, 39.19, 33.68, and 22.75 kD). Line 28 exhibited 28 bands under control and 26 bands under salinity. It had three bands present under control only (44.17, 28.40 and 13.16 kD) and one band present

under salinity only (22.25 kD). Line 1H showed 26 bands under control and 25 bands under salinity. It showed one band under control only (22.25kD). Line 2H is characterized by 28 bands under control and 22 bands under salinity. It had 12 bands under control only (112.24, 106.4, 76.16, 58.25, 42.51, 38.36, 33.68, 24.75, 24, 22.25, 21.5 and 13.16 kD). It exhibited only six bands under salinity (50.3, 39.19, 34.12, 20.5, 24.89 and 11.92 kD). Line 3H showed 24 bands under control and 26 bands under salinity. It had two bands under salinity only (24.75, and 22.25 kD). Meanwhile, Line 4H had 26 bands under both control and salinity stress. It did not change in protein bands, but it contains high density bands (38.63, and 25.76 kD) under control compared with salinity stress.

Table (3) and Figure (2) contain four lowest salinity tolerant lines (1L, 2L, 3L, and 4L Lines). 1L showed only three bands under control (27.92, 26.74, and 25 kD). This line had 22 bands under control and 19 bands under salinity. Also, 2L showed 22 bands under control and 19 bands under salinity. It contained three bands only under control (26.74, 25 and 11.44 kD). Line 3L had 21 bands under control and 18 bands under salinity. It had only five bands under control (41.67, 32.05, 27.92, 23.76, and 11.44 kD). It contained only two bands under salinity (26.74, and 25 kD). Line 4L showed 18 bands under control and 16 bands under salinity. It had four bands under control (91.1, 26.74, 25, and 21.9 kD) but under salinity it had only two bands (33.82, and 11.44 kD).

All genotypes varied in protein band number affected by salinity stress either by appearance or absence on gel. The results of SDS-PAGE analysis showed that several new proteins, which are synthesized in response to salinity stress as stress proteins were expressed in the appearance of certain bands. These results are in agreement with those obtained by Oliver and Bewely (1984) who found that the new proteins resulting from the environmental alterations have been reported as stress proteins or shock proteins. Most of these proteins appear as immediate response by organisms to an altered environment such as heat shock, osmotic stress, drought stress, and/or salt stress. Fahmy *et al.* (2002) showed accumulation of some new polypeptide bands related to salt treatment and that they could be considered as marker proteins for salt adaptation. El-Sawy *et al.* (2003) reported that some protein bands disappeared at four NaCl concentrations among distinctive genotypes and other bands were more informative as indicators for salt tolerance.

2. RAPD fingerprints

The Randomly Amplified Polymorphic DNA (RAPD) technique has been used in many different applications involving the detection of DNA sequence polymorphisms (Carlson *et al.*, 1991).

PCR used in the present study to examine the banding pattern in H and L lines to. Out of 28 primers used, only 16 primers were able to show bands (Fig. 3, 4 and 5). Of these 16 primers primer B5 had unique markers to Line 28 (sensitive to salinity) and the four sensitive lines (1L, 2L, 3L, and 4L) at 900 bp (Fig. 5), it's may be fragment sensitive to salinity.

Table 2. Electrophoresis banding patterns of kernels protein of seven wheat lines (3 parents and 4 salinity tolerant lines)

Bands no.	M.W. kD	Sakha 8		Line 25		Line 28		1H		2H		3H		4H	
		Con.	12000 ppm	Con.	12000 Ppm	Con.	12000 Ppm	Con.	12000 ppm	Con.	12000 ppm	Con.	12000 ppm	Con.	12000 ppm
1	112.24	+	+	+	+	+	+	+	+	+	-	+	+	+	+
2	106.04	+	+	+	+	+	+	+	+	+	-	+	+	+	+
3	86.12	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	76.16	+	+	+	+	+	+	+	+	+	-	+	+	+	+
5	63.55	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	58.25	+	+	+	+	+	+	+	+	+	-	+	+	+	+
7	50.30	+	+	+	+	+	+	+	+	-	+	+	+	+	+
8	44.17	-	+	-	+	+	-	-	-	-	-	-	-	-	-
9	42.51	+	+	+	-	-	-	-	-	+	-	-	-	-	-
10	39.19	+	+	-	+	+	+	+	+	-	+	-	-	-	-
11	38.36	-	-	-	-	-	-	-	-	+	-	+	+	++	+
12	34.12	+	+	+	-	-	-	-	-	-	+	-	-	-	-
13	33.68	-	-	-	+	+	+	+	+	+	-	+	+	+	+
14	31.48	+	+	+	-	+	+	-	-	+	+	-	-	-	-
15	28.40	-	-	+	+	+	-	+	+	+	+	+	+	+	+
16	27.52	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17	26.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	25.76	+	+	+	+	+	+	+	+	+	+	+	+	++	f
19	24.75	+	+	+	+	+	+	+	+	+	-	-	+	+	+
20	24.0	-	+	+	+	+	+	+	+	+	-	+	+	+	+
21	23.25	-	-	+	+	+	+	+	+	+	+	+	+	+	+
22	22.75	-	-	-	+	+	+	+	+	+	+	+	+	+	+
23	22.25	-	-	+	-	-	+	+	-	+	-	-	+	+	+
24	21.50	-	-	+	-	-	-	-	-	+	-	-	-	-	-
25	20.5	+	+	+	+	+	+	+	+	-	+	+	+	+	+
26	20.00	-	-	-	-	-	-	-	-	+	+	-	-	-	-
27	18.40	+	+	+	+	+	+	+	+	+	+	+	+	+	+
28	17.86	+	+	+	+	+	+	+	+	+	+	+	+	+	+
29	17.32	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30	16.84	+	+	+	+	+	+	+	+	+	+	+	+	+	+
31	15.43	+	+	+	+	+	+	+	+	+	+	+	+	+	+
32	14.89	+	+	+	+	+	+	+	+	-	+	+	+	+	+
33	13.16	-	-	-	-	+	-	-	-	+	-	-	-	-	-
34	11.92	+	+	+	+	+	+	+	+	-	+	+	+	+	+
		23	25	28	26	28	26	26	25	28	22	24	26	26	26

- =absent F = faint + = present ++ =high density

Table 3. Electrophoresis banding patterns of kernels protein of seven wheat lines(3 parents and 4 salinity sensitive lines)

Bands no.	M.W. kD	Sakha 8		Line 25		Line 28		1L		2L		3L		4L	
		Con.	12000 ppm	Con.	12000 ppm	Con.	12000 ppm	Con.	12000 ppm	Con.	12000 ppm	Con.	12000 ppm	Con.	12000 ppm
1	103.55	-	-	-	+	+	+	+	+	+	+	+	+	+	+
2	97.33	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	91.10	+	+	+	-	-	-	-	-	-	-	-	-	+	-
4	65.55	-	-	-	+	+	+	+	+	+	+	+	+	+	+
5	58.25	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	52.95	-	-	+	+	+	+	+	+	+	+	-	-	-	-
7	45.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	41.67	+	+	+	-	+	+	-	-	-	-	+	-	-	-
9	37.23	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	33.82	+	+	-	+	+	+	+	+	+	+	+	+	-	+
11	32.64	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	32.05	+	-	-	+	+	+	+	+	+	+	+	-	-	-
13	29.69	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	29.1	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	27.92	+	+	+	-	+	+	+	-	-	-	+	-	-	-
16	26.74	-	+	+	+	-	+	+	-	+	-	-	+	+	-
17	25.0	-	-	+	-	+	+	+	-	+	-	-	+	+	-
18	23.76	+	+	-	-	+	+	+	+	+	+	+	-	-	-
19	21.9	+	+	+	+	+	+	+	+	+	+	+	+	+	-
20	21.28	+	+	+	+	+	+	+	+	+	+	+	+	+	-
21	19.42	+	+	+	+	+	+	+	+	+	+	+	+	+	+
22	17.7	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	15.6	+	-	+	+	+	+	+	+	+	+	+	+	+	+
24	14.4	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25	11.44	+	+	-	+	-	-	-	-	+	-	+	-	-	+
total		20	19	19	20	22	23	22	19	22	19	21	18	18	16

- =absent F = faint + = pres

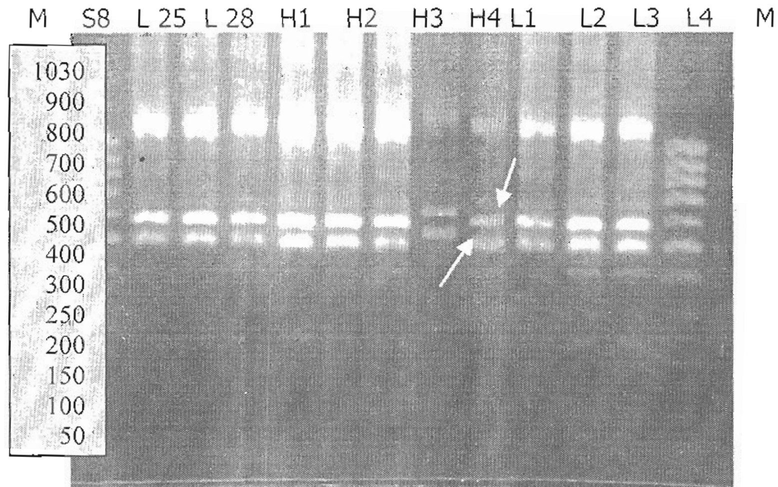


Figure 3. DNA polymorphism of the eleven genotypes using randomly amplified polymorphic DNA-PCR with primer Amersham 3

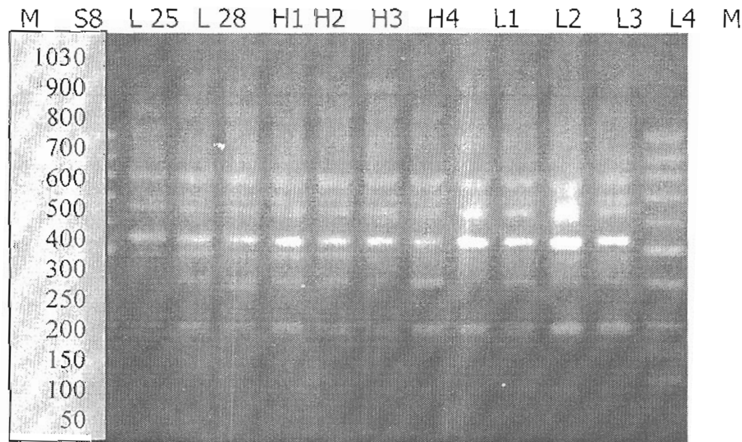


Figure 4. DNA polymorphic of the eleven genotypes using randomly amplified polymorphic DNA-PCR with primer B1

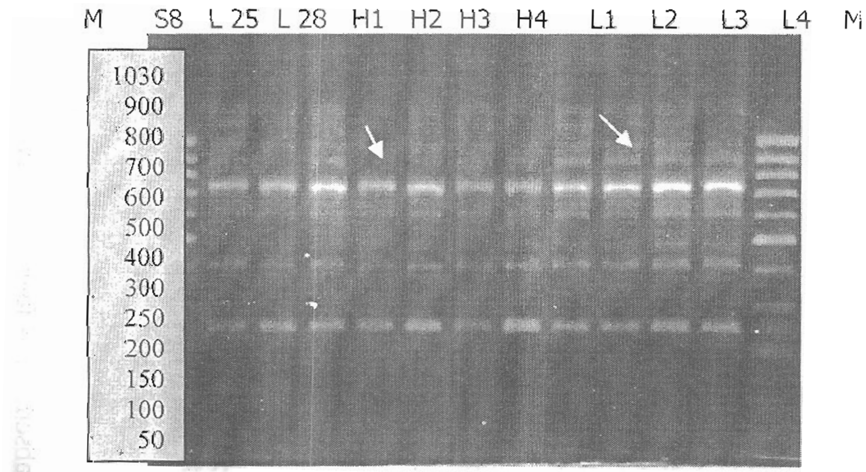


Figure 5. DNA polymorphic of the eleven genotypes using randomly amplified polymorphic DNA-PCR with primer B5

Table 4. Analysis of` polymorphism among eleven Lines by using sixteen primers.

Primers	No. of bands											Total bands	MM	PM	PM%
	S8	25	28	1H	2H	3H	4H	1L	2L	3L	4L				
A7	3	4	4	4	4	4	4	4	4	5	5	5	3	2	40
A11	4	4	5	5	5	4	5	5	4	5	5	6	2	4	66.7
B1	4	7	6	7	7	7	7	7	7	7	6	8	4	4	50
B3	5	6	7	6	4	5	5	4	6	6	6	8	3	5	62.5
B5	4	3	6	3	4	3	3	6	6	6	6	6	3	3	50
B9	3	3	3	3	3	3	4	4	4	4	4	4	3	1	25
B10	8	7	3	8	8	7	8	5	5	5	4	8	2	6	75
B13	10	9	10	9	9	10	10	10	10	10	10	10	9	1	10
B17	6	5	5	6	8	4	7	6	6	7	7	8	3	5	62.5
D5	5	4	4	5	5	5	5	5	3	4	5	5	2	3	60
A.S 1	8	7	9	7	9	8	8	8	5	7	7	13	2	11	84.6
A.S2	0	1	2	1	2	1	4	1	0	2	3	5	0	5	100
A.S3	3	3	3	4	4	4	3	3	3	3	3	6	3	3	50
UBC2	4	5	4	5	5	5	4	6	4	6	5	7	1	6	85.7
UBC61	3	3	3	3	3	3	3	2	2	2	2	3	2	1	33.3
UBC82	3	3	3	8	5	5	6	6	4	4	4	9	3	6	66.7
Total	73	74	77	84	85	78	86	82	73	83	82	111	45	66	
Average												6.94	2.81	4.13	57.63

S8= Sakha 8, 25= Line 25, 28= Line 28, high salinity tolerant genotypes= 1H, 2H, 3H, and 4H low salinity tolerant genotypes= 1L, 2L, 3L, and 4L A.S.= Amer Sham MM= Monomorphic PM= polymorphic

RAPDs analysis of 11 bread wheat genotypes produced multiple band profile with a number of amplified DNA fragments ranged from 3 to 13 (Table 4). The total number of fragments produced by 16 primers were 111 with average number of 6.94 fragments/primer, while, the number of monomorphic fragments ranged from 0 to 9

with an average of 2.81 fragments/primer, and the number of polymorphic fragments ranged from 1 to 11 with average number of 4.13 fragments/primer. The average percentage of polymorphism caused by the sixteen primers was 57.63. The maximum percentage was 100 with primer AmerSham2 and the minimum percentage was 10 with primer B13. The maximum number of 13 amplicons was amplified with primer AmerSham1. The minimum number of fragments was 1 amplified with primer UBC61. Other workers found similar results He *et al.* (1992) showed that out of 65 primer combinations used for PCR amplifications over 38% produced readily detectable and reproducible DNA polymorphisms between the spring wheat line 50-852 and winter wheat variety Clark.

Unique markers revealed by RAPD:

RAPD technique was employed to detect unique markers which could discriminate between the eleven wheat genotypes. Unique markers are defined as bands that are present in one line and absent in the other.

Twelve primers out of 16 primers detected single positive or negative unique markers (Table 5). The highest number of unique markers was observed in Line 25, 3H, and 4H, which gave three unique markers. Sakha 8 and 2L gave two unique markers. Line 28, 1H, and 1L gave one unique markers. Sakha 8 gave one negative unique marker with primer A7 (570 bp) and primer B1 (400bp, and 300 bp). Line 25 scored one unique marker positive, (900, and 650 bp) and negative (700 bp). Line 28 gave three unique bands, positive with primer A11 (900 bp) and primer B3 (1200 bp), and negative with primer B10 (590 and 500 bp). 1H gave one positive unique marker (950 and 500 bp). 3H scored three negative unique bands with primer A11 (600 bp), B17 (500 and 300 bp), and Amersham1 (1031bp). 4H gave two positive unique markers with Amersham2 (550bp), and Amersham3 (650 and 550bp), and negative band (500bp). This line gave also one negative unique marker with UBC2 primer (1500 bp) whereas, 1L gave one negative unique marker (850bp) with B13 primer, while 2L scored two negative unique markers with primer D5 (1031, 600 bp) and primer Amersham1 (750 bp) as shown in Table (5). Other workers found similar results. Mehboob-ur-Rahman *et al.* (2004) detected only one band (680pb) as a marker for salinity tolerance.

Table 5. Unique markers revealed by RAPD

Ser. No.	Primers	Line	Marker type	Molecular size
1	A7	Sakha 8	-	570
2	A11	Line 28	+	900
		3H	-	600
3	B1	3L	+	600
		Sakha 8	-	400
		Sakha 8	-	300
4	B3	Line 28	+	1200
		1L	-	850
5	B10	Line 28	-	590
		Line 28	-	500
6	B17	3H	-	500
		3H	-	300
7	D5	2L	-	1031
		2L	-	600
8	Amr Sham 1	3H	-	1031
		Line 25	+	900
		2L	-	750
		Line 25	-	700
		Line 25	+	650
9	Amr Sham 2	4H	+	550
10	Amr Sham3	4H	+	650
		4H	+	550
		4H	-	500
11	UBC2	4H	-	1500
12	UBC82	1H	+	950
		1H	+	500

Genetic similarity and cluster analysis:

The RAPD data developed by all primers of this study were used to estimate the genetic similarity among the eleven lines. The genetic similarity matrix based on possible pairs of cultivars ranged from 15.7% between S8 and 4L to 92.3% between 3L and 4L (Table 6). The highest genetic similarity observed between 4L and 3L was 92% followed by 3L and 2L with 88%, while the lowest genetic similarity value was shown between Sakha 8 and 4L with 15 % followed by 3L and Sakha 8 (17%).

Table 6. Matrix of similarity estimate (%) among the eleven wheat genotypes based on RAPD analysis.

Ser no.	Genotype	Sample matching measure										
		S8	line 25	Line 28	1H	2H	3H	4H	1L	2L	3L	4L
1	Sakha 8											
2	Line 25	56.9										
3	Line 28	43.6	67.4									
4	1 H	36.7	61.9	71.0								
5	2 H	30.6	52.4	62.8	83.1							
6	3 H	26.7	44.6	56.0	73.4	83.7						
7	4 H	23.3	40.9	50.9	67.3	75.9	81.6					
8	1 L	20.7	37.3	50.3	60.4	70.3	76.3	82.9				
9	2 L	17.5	32.3	45.4	52.3	60.1	66.4	72.7	87.3			
10	3 L	17.1	32.2	44.3	51.6	59.8	65.5	72.2	84.8	88.2		
11	4 L	15.7	29.0	41.8	47.6	56.1	61.1	69.0	79.5	84.1	92.3	

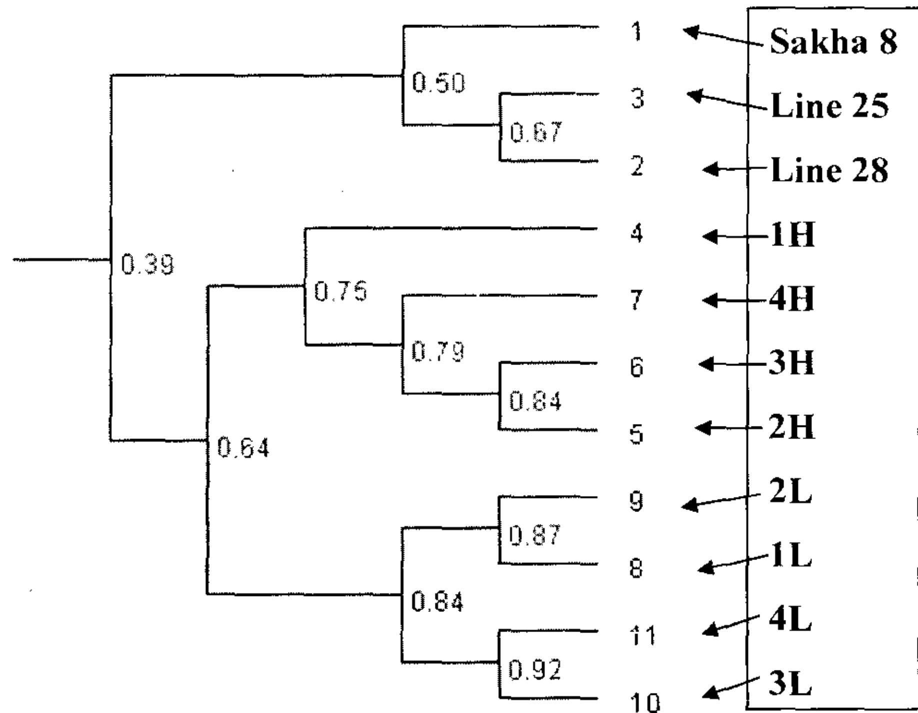


Fig 6. Dendrogram for the eleven wheat genotypes constructed from RAPDs data using Unweighted Pair Arithmetic Average (UPGMA) and similarity matriced computed according to Dice coefficient.

The dendrogram based on genetic similarities (Fig. 6) classified the eleven genotypes into two main clusters, of the parents which were separated to sub cluster

Sakha 8 and lines 25 and 28. The other sub cluster contained eight lines, which were separated to two groups, the first group contained the high tolerant lines, which is divided into 1H and a group containing 2H, 4H and 3H. The second group of the second sub cluster included two groups, the first was 1L and 2L and the second group was 3L and 4L. These results agree with Barakat *et al.* (2000) using RAPD analysis, and they found that the genetic similarity among six wheat cultivars ranged from 41% t0 84%, and the wheat genotypes were classified into three clusters, Giza cultivars, Sakha 69 and Gemmiza 1 cultivars and Sohag1. They reported also that also RAPD technique might help in studying genetic relationship among different wheat cultivars. Kudriavtsev *et al.* (2003) calculated the similarity indices among 64 durum wheat cultivars using pedigree and RAPD analysis. They found a correspondence between the two approaches for estimation of genetic diversity.

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التكبير العشوائى المتعدد الصور للحمض النووى دنا (DNA) والتفريد الكهربى لبروتين سلالات القمح المتحملة للملوحة المستولدة من الجيل الرابع (R_4)

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الهدف من إجراء هذا البحث هو دراسة الاختلافات الوراثية بين أعلى ٤ سلالات متحملة للملوحة وأقل ٤ سلالات تحملا (حساسة للملوحة) منتجين من تباين الكلونة بمزارع الأنسجة (R_4) مقارنة بآبائهم (سخا٨ متحمل للملوحة وسلالتين ٢٥ و٢٨ حساستين للملوحة) على المستوى الجزيىء باستخدام معلمين جزيئين بواسطة التفريد الكهربى للبروتين السلالات والآباء تحت مستويين من تركيز الملوحة (الكنترول ومستوى ١٢٠٠٠ جزء فى المليون). وكذلك التكبير العشوائى المتعدد الصور للحمض النووى دنا (DNA) باستخدام ٢٨ بادىء عشوائى. لوحظ بالتفريد الكهربى للبروتين ظهور واختفاء بعض وحدات البروتين نتيجة تأثير الملوحة وذلك فى جميع السلالات والآباء أيضا. ومن بين ٢٨ بادىء عشوائى استخدموا فى التكبير العشوائى المتعدد الصور للحمض النووى دنا لم يستجب إلا ١٦ بادىء فقط و أظهر البادئ B5 حزمة واحدة للأب (سلالة ٢٨) وكذلك للأربعة سلالات الحساسة عند bp٩٠٠، وزادت نسبة الحزم المتباينة بنحو ٥٧,٦٣%عن الحزم غير المتباينة، وقسمت السلالات والآباء من حيث درجة القرابة إلى مجموعتين رئيسيتين الأولى ضمت الآباء و الثانية ضمت السلالات الناتجة من مزارع الأنسجة للأجنة غير الناضجة وهذا يدل على أن السلالات الناتجة مختلفة عن الآباء الناتجة ومتباعدة وراثيا. وكان أعلى درجة قرابة ٩٢% وجدت بين السلالتين 3L, 4L بينما أقل درجة قرابة (١٥,٧%) وجدت بين أحد الآباء (S8) واحدى السلالات الناتجة (4L).

وكان من أهم نتائج هذا البحث هو أن التحليل بالمعلمات الجزيئية أظهر ارتباطا مع المحصول ومكوناته.