Genetic variation for salt tolerance in some bread and pasta wheat genotypes

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Reda E.A. Moghaieb*; Neveen B.Talaat, Abdel-Hadi A. Abdel-Hadi*, Sawsan S. Youssef* and Ahmed M. El-Sharkawy***

*Department of Genetics and Genetic Engineering Research Center, Faculty ofAgriculture, Cairo University, Giza, Egypt.

**Department of Botany-Plant Physiology Division, Faculty of Agriculture, Cairo University, Giza, Egypt

*-Corresponding author

ABSTRACT

In order to investigate the influence of genetic makeup on salt tolerance in wheat, seedlings of seven T. aestivum and two T. durum cultivars were subjected to salt stress for two weeks. Leaf osmotic adjustment, total sugars, free amino acids and proline under salt stress were studied. The concentration of total sugars, total free amino acids and free proline were increased by salinity. Salt stress decreased leaf osmotic potential in all cultivars. The difference in osmotic adjustment between the cultivars was correlated with the concentrations of minerals examined such as Na+ and K+. The salt tolerance in the T. aestivum cultivar Gemmeiza 10, and in T. durum cultivars Sohag and Beni Sweif is due to higher ability to maintain osmotic potential of the cells than the other cultivars by increases in osmoticum concentration under salt stress. The cultivars Gemmeiza10, Sohag and Beni Sweif showed higher peroxidase band intensity under salinity stress compared with the other cultivars used. The genetic polymorphism between the cultivars was detected by RAPD and SSR analyses. Eighty-two out of 118 RAPD markers detected were polymorphic (69.5%) and 42 out of 59 SSR alleles were polymorphic (71%) and can be considered as useful markers for the nine wheat cultivars. The genotype specific markers (RAPD and SSR) for each cultivar were determined. Eighteen RAPD markers and thirteen SSR markers generated were found to be genotype-specific. Seven markers distinguished the cultivar Beni Sweif, six markers for the cultivar Sohag and two markers for the cultivar Gemmeiza 10. These markers could be considered as being genetic markers associated with salt tolerance in the three wheat genotypes and help in marker-assisted selection breeding program.

Keywords: osmotic adjustment; RAPD and SSR marker; salt tolerance, wheat genomes.

INTRODUCTION

Salinity is one of the major factors responsible for low yield and restricted economic utilization of land resources both in arid and semi arid regions of the World (Ghassemi *et al.*, 1995). The progressive salinization of soil was estimated at around 20% of irrigated land (Ghassemi *et al.*, 1995). Approximately 20 mha of land deteriorates to zero production each year (Malcolm, 1993) mainly due to salinization. Thus, with continuous land losses and increasing population, there is tremendous pressure to avoid food shortages. Wheat is one of the most abundant sources of energy and nourishment

for mankind. Ninety-five percent of the cultivated wheat is of the hexaploid type used for the preparation of bread and other baked products and the remaining 5% is durum (tetraploid) wheat, which is used essentially for making pasta and macaroni (Bushuk 1998). Wheat is classified as a semi tolerant crop to salinity. One way to alleviate the problem is the breeding of salt tolerant genotypes that perform better than current sensitive varieties under moderate to high salinity stress. Identifying genotypes that are tolerant to saline conditions is a practical and relatively simple way of improving crop yield and profitability on these difficult soils. Different physiological traits such as potassium selectivity, exclusion and/or compartmentation of sodium, and chloride ions, balance of nitrate and chloride, osmotic adjustment and the accumulation of organic solutes have all been related to the salt tolerance of genotypes of different species (Weimberg, 1987; Yeo *et al.,* 1990). Growing tolerant genotypes on soils with salinity reflects the shift to a strategy of 'tailoring the plants to fit the soil' in contrast to the older strategy of 'tailoring the soil to fit the plant'. Genotype tolerance to salinity may be considered as a substitute for amendments/ reclamation on moderately saline soils and a supplement to amendments in strongly saline ones. Genetic variability within a species offers a valuable tool for studying mechanisms of salt tolerance. The analysis of genetic variation and relatedness in germplasm are of great value for genetic resources conservation and plant breeding programs to determine the best crosses between different genotypes. Over the years, the methods for assessing genetic diversity have ranged from classical strategies such as morphological analysis to biochemical and molecular techniques (Demissie *et al.,* 1998). Several molecular approaches have been used to identify, diagnose, delimit species and assess phylogenetic relationships between different cultivars, among many others, three molecular methods, random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and more recently DNA sequencing, have been the most extensively applied.

The random nature of RAPDs (Williams *et al.*, 1990) means they complement isozyme variation, which only reflects differences in protein-coding genes. Although less reliable than allozymes for the estimation of genetic parameters in populations of out-crossing diploids (Liu and Furnier, 1993), they can detect more polymorphism. Usefulness of RAPDs in several plant species has been demonstrated for variety identification (Hu and Quiros, 1991; Lazaro and Aguinagalde, 1996), gene bank management (Kresovich *et al.*, 1992), taxonomic studies (Demeke *et al.,* 1992) and gene diversity evaluation (Margale *et al.*, 1995). Liu and Furnier (1993) demonstrated that RAPD markers are very useful for discriminating individual genotypes. SSRs offer a potentially attractive combination of features that are useful as molecular markers. First, SSRs have been reported to be highly polymorphic and thus highly informative in plants, providing many different alleles for each marker screened, even among closely related individuals (Akkaya *et al.,* 1992; Saghai- Maroof *et al.,* 1994). Second, SSRs can be analyzed by a rapid, technically simple, and inexpensive PCR-based assay that requires only small quantities of DNA. Third, SSRs are co-dominant and simple Mendelian segregation has been observed. Finally, SSRs are both abundant and uniformly dispersed in both human (Weber, 1990) and plant genomes (Lagercrantz et al., 1993; Wang *et al.,* 1994, Akkaya *et al.,* 1995). The primary disadvantage of SSRs as molecular markers is the cost and research effort required to clone and sequence SSR-containing DNA fragments from the plant species of interest.

Keeping in view the above scenario, the present study has been planned to investigate variation among nine wheat genotypes for their tolerance to different salinity concentrations and to identify the physiological and biochemical processes that make a plant tolerant to salinity. Also to determine the genetic markers related to salt tolerance in wheat cultivars under investigation at isozyme, SSR and randomly amplified polymorphic DNA (RAPD) levels.

MATERIALS AND METHODS

Plant material and culture conditions

The experiment reported here was conducted in the Genetic Engineering Research Center, Faculty of Agriculture, Cairo University, Egypt. In the present work, nine Egyptian wheat cultivars namely Sahel-1, Giza-160, Giza-168, Gemmeiza -7, Gemmeiza -9, Gemmeiza -10, Sids-1, Beni-Sweif and Sohag, were used. Seeds were planted in plastic pots (3 L) each containing a mixture of sandy soil and peat moss (1:1 v:v). Seedlings were irrigated daily with 400 mL of one tenth of the MS solution and the soil water tension was maintained at $\leq$ 60 k P_a. After 30 days from planting, the plants were subjected to salt stress by the addition of 0, 50, 100, and 150 mM NaCl to the irrigation solution for 15 days. The temperature was 25°C and the photosynthetically active radiation was 2743 µ mole m^{-2} s^{-1} (photosynthetic active radiation PAR). There were five replications per NaCl treatment and the control (no treatment with NaCl).

Determination of leaf water relations

Leaf samples were frozen in a liquid nitrogen, and stored at -20°C. Tissues were thawed and centrifuged at 1,200 xg for 25 min at 4°C to extract the cell sap. Osmotic potential (ψ_s) of the cell sap was measured using a vapor pressure osmometer (model 5,500, Wescor, Logan, UT, USA). Osmotic adjustment (OA) was calculated as the differences in (ψ_s) between salinized and control plants.

Chemical analysis

Random samples of each treatment were used to determine the following chemical analyses. Nitrogen and phosphorus were determined according to Pregl (1945) and Jackson (1967), respectively. Potassium and sodium were determined using the Flamephotometer. Calcium was determined by using atomic absorption spectrophotometer. Total sugars, total free amino acids and free proline were determined according to A.O.A.C. (1965), Moore and Stein (1954) and Bates *et al.* (1973), respectively.

Isozyme analysis

Isozyme extraction was performed using control plant (0 mM NaCl as well as leaf tissue from NaCl treated plants. Tissue (400 mg) was ground in 2 ml extraction buffer (0.1% (w/v) Tris-citric acid, pH 7.5; 1% (w/v) polyvinyl pyrolidone (PVP); 0.1% (w/v) ascorbic acid and 0.1% (w/v) cysteine) and centrifuged at 5333 xg (JS - 5.2 roter), at 4 °C for 5 min. Twenty µl of extracted samples were used for electrophoresis on polyacrylamide gel (SDS-PAGE) according to the method of Stegman *et al.*(1983) using Pharmacia electrophoresis apparatus (GE-4).

Peroxidase detection

Peroxidase was detected by incubating the gel in darkness for one hour at 37°C in a mixture of 15 ml of 10% benzidine (in 95% ethanol); 85 ml of 1mM potassium acetate and 1 ml of 1% H_2O_2 (pH 4.7). After the incubation period the gel was rinsed in distilled water and fixed in 50% glycerol for one hour.

Molecular analyses

DNA extraction

Total genomic DNA was isolated using the method described in Rogers and Bendich (1985).

RAPD analysis

PCR reactions were conducted using arbitrary 10-mer primers (Operon Technology, Inc., Alameda, CA, USA). The names and sequences of the primers that give clear bands are as follows:

Primer	Sequence
OPB-07	5`-GAAACGGGTG -3`
OPC-05	5`-GATGACCGCC -3`
OPD-05	5`- TGAGCGGACA -3`
OPG-12	5`-CAGCTCACGA -3`
OPM-05	5`- GGGAACGTGT -3`
OPN-04	5`- GACCGACCCA -3`
OPN-10	5`-ACAACTGGGG -3`
OPN-13	5`-AGCGTCACTC - 3`
OPQ-12	5`- AGTAGGGCAC-3`
OPQ-14	5`-GGACGCTTCA -3

The reaction mixture (20 µl) contained 10 ng DNA, 200 µM dNTPs, 1 µM primer, 0.5 units of Red Hot Taq polymerase (AB-gene Housse, UK) and 10-X Taq polymerase buffer (AB-gene Housse, UK). Samples were heated to 94°C for 5 min and then subjected to 35 cycles of 1 min at 94°C; 1 min at 35°C and 1 min at 72°C. The amplification products were separated in 1% (w/v) agarose gel in 1 x TBE buffer and visualized by staining with ethidum bromide. Reproducibility of DNA profiles was determined by replicating all RAPD reactions at least three times. Variations among wheat genotypes across the primers used in the present. study were evaluated from pairwise comparison for the proportion of shared bands amplified (Nei, 1987).The similarity coefficients were calculated by using statistical software package STATISTICA_SPSS (Stat Soft Inc.).

SSR analysis

Five SSR markers described by Brown *et al.* (1996) and Kong *et al.* (2000) were used for genotyping assays. Primers names, sequences and corresponding annealing temperatures are listed as follows:

Table (1): Types and sequences of the SSR loci and annealing temperature for PCR reaction.

Lotus	Type of SSR (s)	Sequence of forward primer	Sequence of reverse primer	Ann Temp.
Xtxp-7	$(CT)_{14}$	ACATCTACTACCCTCTCACC	ACATCTACTACCCTCTCACC	50
Xtxp-8	(TG)31	ACAT CTACT AC CCT CTCACC	ACACATCGAGACCAGTTG	50
Xtxp-10	(TG)14	ATACTATCAAGAGGGGAGC	AGTACTAGCCACACGTCAC	50
Xtxp-12	(CT)22	ATAT GGAAGGAAGAAGC C GG	AACACAACAT GCAC GCAT G	55
Xtxp-19	$(AG)_5$ +$(AG)_{10}$	ATACTATCAAGAGGGGAGC	AGTACTAGCCACACGTCAC	55

Polymerase chain reaction (PCR)

The polymerase chain reaction was carried out in a Biometra thermal cycler using primers listed in Table 1. The PCR reaction mix includes the following: DNA, 10 ng/µl; 0.5 units of Red Hot Taq polymerase (AB-gene Housse, UK) and 10-X Taq polymerase buffer (AB-gene Housse, UK). 10 mM dNTPs; 50 mM MgCl2; 10 µM each of forward and reverse primers. The PCR profile starts with 95°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min extension at 72°C for 2 min. A final extension 72°C for 7 min was included. The amplification products were separated in 2% (w/v) agarose gel in 1 x TBE buffer and visualized by staining with ethidum bromide.

Data analysis

All the genotypes were scored for the presence and absence of the SSR bands. And the data were entered into a binary matrix as discrete variables, 1 for presence and 0 for absence of the character and this data matrix was subjected to further analysis. The Excel file containing the binary data was imported into NT Edit of NTSYS-pc 2.02J. The 0/1 matrix was used to calculate similarity as DICE coefficient using SIMQUAL subroutine in SIMILARITY routine. The resultant similarity matrix was employed to construct dendrogram using Sequential Agglomerative Hierarchical Nesting (SAHN) based Unweighted Pair Group Method with Arithmetic Means (UPGMA) to infer genetic relationships and phylogeny.

RESULTS

Salinity stress

In the present study, all the parameters of leaf water relations decreased with increasing NaCl concentration in the culture media (Table 2). The osmotic potential (ψ_s) of the salt treated plants decreased with increasing NaCl concentration and the decrease was more pronounced in Gemmeiza-10. Osmotic adjustment (O.A.) increased with NaCl concentration, and was greater in Gemmeiza -10 followed by Sohag then Beni- Sweif and lowest in Gemmeiza -7. The difference in ψ_s and O.A. seemed to be related to the accumulation of sodium ions. The difference in the ability to maintain the osmotic potential under salt conditions between the wheat cultivars used in the present study reflects the differences in their genetic backgrounds.

Table (2): Osmotic potential (ψ_s) and osmotic adjustment (O.A.) in nine wheat cultivars under salinity stress.

	NaCl (mM)	Wheat genotypes								
		Sahel-1	Giza 160	Giza 168	Gemmeiza 7	Gemmieza 9	Gemmieza 10	Sids 1	Beni Sweif	Sohag
ψ_s (M.Pa)	0	-1.73	-1.24	-1.24	-1.49	-1.73	-1.98	-1.98	-1.73	-1.73
	50	-1.98	-1.49	-1.73	-1.73	-1.86	-2.21	-2.31	-2.13	-2.23
	100	-2.23	-1.88	-1.98	-1.81	-2.41	-2.82	-2.47	-2.97	-3.22
	150	-2.73	-2.35	-2.35	-2.23	-3.47	-5.71	-2.97	-4.96	-5.21
O.A.	0									
	50	0.25	0.25	0.29	0.24	0.13	0.23	0.33	0.4	0.5
	100	0.5	0.64	0.74	0.32	0.68	0.84	0.49	1.24	1.49
	150	1.0	1.11	1.11	0.74	1.74	3.73	0.99	3.23	3.48

The concentration of total sugars, total free amino acids and free proline of different wheat genotypes was increased by salinity (Table 5). Gemmeiza 10, Sohag and Beni-Sweif genotypes accumulated the highest concentrations, while Giza 160 and Sids 1 maintained the lowest concentrations followed by Sahel 1, Gemmeiza 9, Gemmeiza 7 and Giza 168 in an ascending order. On the other hand, salinity decreased nitrogen, phosphorus, potassium and calcium concentrations as well as K^+: Na^+ ratio of all the tested wheat genotypes (Table 3). The maximum ratios and concentrations were observed in Gemmeiza 10, Sohag and Beni-Sweif genotypes and the minimum ones were observed in the case of Giza 160 and Sids 1. It means that these genotypes differed in their performance against salinity and these variations may be exploited for the development of a tolerant genotype. Gemmeiza 10 and Sohag genotypes proved to be the best of all; they have maintained a higher accumulation of organic metabolites and inorganic ions (Tables 3 and 5). Salinity stress increased band intensity of the salt treated wheat plants much higher than the control plants in all cultivars. The increase in proxidase level increased with increasing salt concentration. The level of band intensities differs between cultivars (Fig. 1).

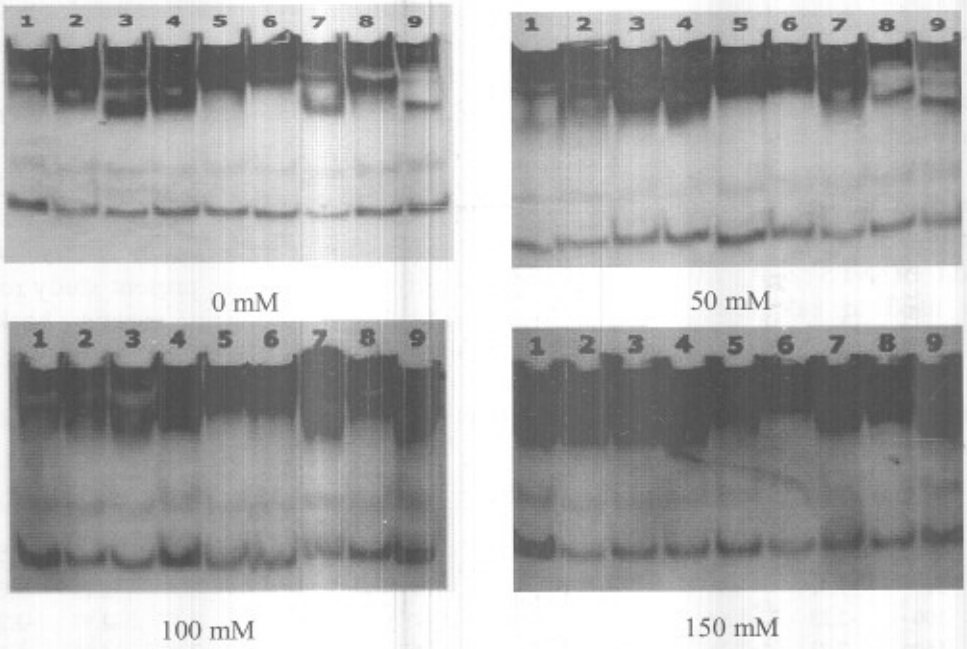

Fig. (1): Peroxidase isozyme profile of the control and salt treated wheat plants under salinity stress. Lanes 1-9: the wheat cultivars Sahel, Giza 160, Giza 168, Gemmeiza 7, Gemmeiza 9, Gemmeiza 10, Sids 1, Beni Sweif and Sohag , respectively.

Table (3): Nitrogen, phosphorus and calcium concentration (mg g^{-1} DW) in the nine wheat cultivars under salinity stress.

	NaCl	Wheat genotypes								
	(mM)	Sahel-1	Giza 160	Giza 168	Gemmeiza 7	Gemmeiza 9	Gemmeiza 10	Sids 1	Beni-Sweif	Sohag
Nitrogen mg g^{-1} DW	0	15.19	14.82	15.88	15.63	15.66	16.40	14.33	16.00	16.00
	50	11.10	10.62	12.61	12.05	11.74	13.66	10.01	13.33	13.31
	100	8.92	9.05	10.94	10.01	10.14	12.00	8.56	11.61	11.83
	150	7.82	7.98	9.05	8.41	8.10	10.89	6.64	9.85	10.00
Phosphorus mg g^{-1} DW	0	1.80	1.75	1.91	1.82	1.82	2.11	1.70	1.90	2.22
	50	1.39	1.29	1.53	1.46	1.43	1.81	1.23	1.70	1.80
	100	1.17	1.10	1.38	1.31	1.21	1.44	1.06	1.31	1.53
	150	0.96	0.91	1.08	1.00	1.00	1.24	0.89	1.11	1.34
Calcium mg g^{-1} DW	0	2.33	2.00	2.80	2.61	2.66	3.31	1.92	3.06	3.46
	50	1.83	150	2.40	2.21	2.29	2.99	1.53	2.78	3.18
	100	1.64	1.36	2.20	2.00	2.10	2.80	1.27	2.55	2.88
	150	1.46	1.20	1.88	1.80	1.80	2.47	1.18	2.19	2.61

Table (4): Na^+ and K^+ concentration (mg g^{-1} DW) in the nine wheat cultivars under salinity stress.

	NaCl	Wheat Genotypes								
	(mM)	Sahel 1	Giza 160	Giza 168	Gemmeiza 7	Gemmeiza 9	Gemmeiza 10	Sids 1	Beni-Sweif	Sohag
Potassium mg g^{-1} DW	0	20.00	18.21	22.11	21.50	22.00	23.71	18.32	23.00	23.50
	50	16.02	15.31	20.11	18.80	19.12	21.80	15.14	21.08	22.00
	100	12.94	12.00	15.04	14.31	14.22	19.03	12.15	17.94	18.89
	150	11.93	10.92	13.64	12.89	13.20	16.88	11.08	15.84	17.00
Sodium mg g^{-1} DW	0	2.03	2.25	1.80	1.88	1.91	1.15	2.31	1.40	1.26
	50	2.68	2.80	2.41	2.50	2.56	1.88	2.86	2.08	2.00
	100	3.47	3.66	3.29	3.31	3.36	2.87	3.60	3.00	2.93
	150	4.26	4.58	4.11	4.03	3.95	3.22	4.60	3.45	3.20
K^+: Na^+ ratio	0	9.85	8.09	12.28	11.43	11.51	20.61	7.93	16.42	18.65
	50	5.97	5.46	8.34	7.52	7.46	11.59	5.29	10.13	11.00
	100	3.72	3.27	4.57	4.32	4.23	6.63	3.37	5.98	6.44
	150	2.80	2.38	3.31	3.19	3.34	5.24	2.40	4.59	5.31

Table (5): The effect of salinity stress on total sugar, free amino acids and proline concentration in nine wheat cultivars.

	NaCl (mM)	Wheat Genotypes								
		Sahel 1	Giza 160	Giza 168	Gemmeiza 7	Gemmeiza 9	Gemmeiza 10	Sids 1	Beni-Sweif	Sohag
Total sugars (mg glucose g^{-1} DW)	0	36.61	35.31	38.64	37.13	37.87	44.91	36.00	41.65	45.10
	50	45.00	40.66	46.98	49.11	48.32	56.14	43.61	53.00	60.11
	100	53.08	51.32	55.31	56.11	56.94	70.01	50.81	62.10	68.34
	150	58.00	54.04	63.10	61.98	61.15	80.10	53.76	71.64	78.81
Total free amino acids (mg g^{-1} DW)	0	1.73	1.50	2.11	2.03	1.96	2.74	1.41	2.70	2.98
	50	2.18	2.00	2.80	2.66	2.57	3.77	1.88	3.61	4.00
	100	3.11	2.50	3.61	3.84	3.66	5.08	2.51	5.14	5.39
	150	3.58	3.00	4.18	4.31	4.40	6.00	2.92	6.12	6.28
Free proline (mg g^{-1} FW)	0	1.19	1.10	1.22	1.21	1.19	1.42	1.13	1.30	1.38
	50	1.41	1.20	1.50	1.53	1.46	1.90	1.32	1.72	1.84
	100	1.73	1.50	1.79	1.91	2.10	2.74	1.57	2.49	2.61
	150	1.78	1.58	2.29	2.20	2.28	3.11	1.67	2.73	2.98

In order to investigate the genetic differences between the cultivars used, the random amplified polymorphic DNA (RAPD) analysis was performed. All primers used in the present study resulted in the appearance of PCR products with a variable number of bands. This study shows that a total of 118 DNA markers were detected among the nine wheat cultivars of which, 82 bands were polymorphic (69.5%) and can be considered as useful RAPD markers for the nine wheat cultivars used in the present study (Fig.2 and Table 6). The highest number of RAPD bands was detected for primers OPM-05, OPN-13 and OPN-10 (18, 16 and 15 bands, respectively), while the lowest was scored for OPB-07 (7 bands).

Table (6): Primers used in RAPD analysis and their number of bands.

Primer name	No. of scorable bands	Polymorphic bands	% of polymorphism
OPB-07	7	6	85
OPC-05	9	7	77
OPG-14	9	6	66
OPM-05	18	14	77
OPO-12	8	4	50
OPN-13	16	13	81
OPD-05	11	3	27
OPQ-14	13	8	61.5
OPN-04	12	8	66
OPN-10	15	13	86.6
Total	118	82	69.5

Table (7): Genotype - specific RAPD – markers.

Genotypes	Markers	Total Marker
Sahel -1	OPN-10(453)	1
Giza -160	OPN-10 (1427), OPQ14 (822), OPN-04 (615)	3
Giza -168	OPQ-14 (1790), OPQ-14 (2726)	2
Gemmeiza -7	-	-
Gemmeiza -9	OPM-05 (373), OP N-13 (388), OPN-04 (1055)	3
Gemmeiza - 10	OPO-12 (780)	1
Sids -1	OPM-05 (242)	1
Beni Sweif	OPC-05 (1238), OPN-10 (1242), OPB-07 (770), OPN-13 (639), OPN-13 (672)	5
Sohag	OPN-13 (1487, 1505),OPN-04 (455)	3
Total		18

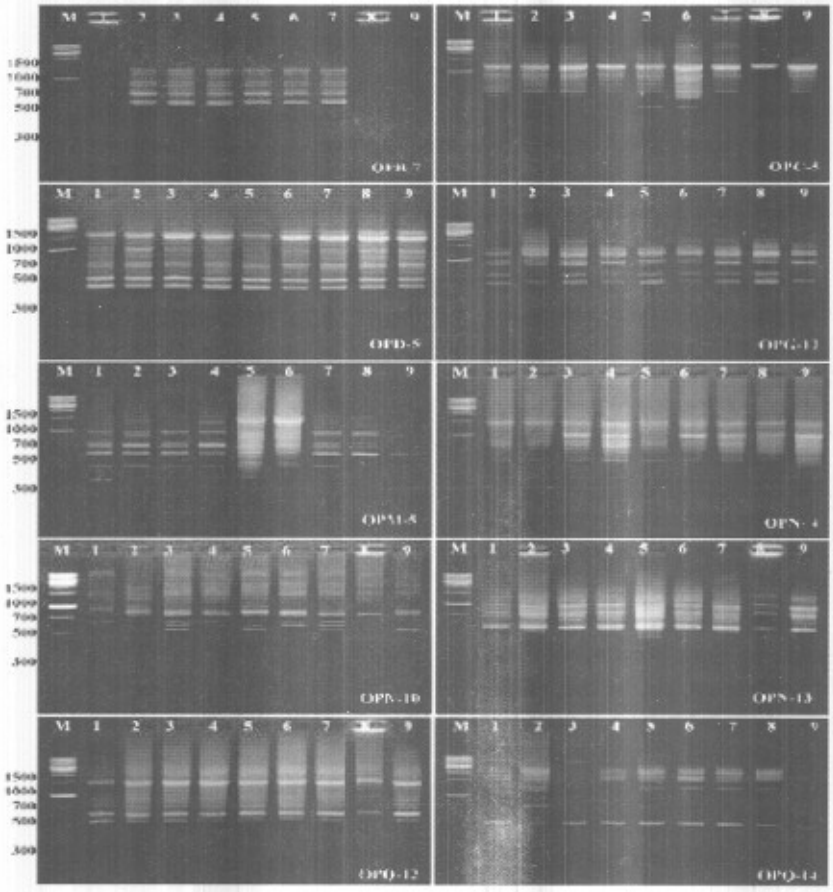

Fig (2):RAPD banding patterns of nine different wheat genotypes using ten selected random primers, M: 1 kbp plus DNA ladder, 1-9: the wheat cultivars Sahel, Giza 160, Giza 168, Gemmeiza 7, Gemmeiza 9, Gemmeiza 10, Sids 1, Beni-Sweif and Sohag , respectively.

The genotype-specific RAPD markers for the different wheat cultivars used in the present study are listed in Table (7). Eighteen out of the eighty two polymorphic RAPD markers generated were found to be genotype-specific (31.4%). The highest number of RAPD specific markers was scored for Beni Sweif (5 markers) while both of Giza-160, Gemmeiza -9 and Sohag scored three markers each. On the other hand, G-168 scored two markers and Sakha-1, Gemmiza -10, Seds-1 scored one marker each. In the meantime, the highest number of RAPD genotype-specific markers was generated for primers OPN-13 (four markers).

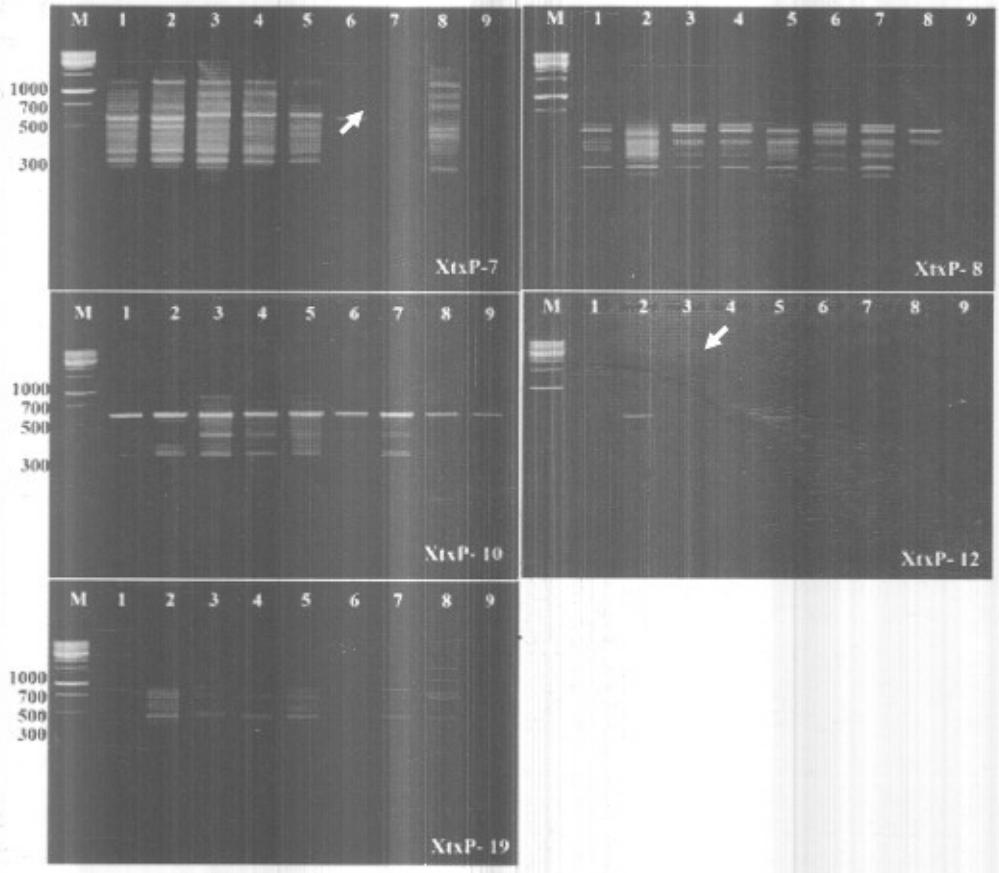

Fig.(3): SSR banding patterns of nine different wheat genotypes using five primers, M: 1 kbp plus DNA ladder, 1-9: the wheat cultivars Sahel, Giza 160, Giza 168, Gemmeiza 7, Gemmeiza 9, Gemmeiza 10, Sids 1, Beni-Sweif and Sohag , respectively.

Five primer pairs flanking dinucleotide simple sequence repeats (CT or AG) were used to investigate the level of polymorphism among the nine wheat genotypes. All primers produced fragments, even when using modified amplification conditions. All primers showed different levels of polymorphism (Figure 3). Most of the alleles were polymorphic, thus revealing 71% polymorphism. The size of the detected alleles produced from using the SSR primer sets ranged from 82- 1620 bp which reflects a large difference in the number of repeats between the different alleles (Table 8). The genotype-specific SSR markers for the different wheat cultivars used in the present study are listed in Table 9). Thirteen out of the forty-two polymorphic SSR markers generated were found t be genotype- specific (30.9%). The highest number of SSR specific markers was scored for Sahel-1 (4 markers) followed by Sohag (3 markers), while Giza-160; Gemmeiza -9 and Beni Sweif scored two markers each. On the other hand, Gemmeiza -10 and Sids-1 scored one marker each.

Table (8): Number of allels, fragment size range and polymorphism detected by SSR loci in the nine wheat genotypes.

Primer	Fragment size (bp)	No Allels	Monomorphic bands	Polymorphic bands	Polymorphism %
XtxP-07	300-1620	14	3	11	78.6
XtxP-08	096-0579	15	4	11	73.3
XtxP-10	105-0969	9	4	5	55.5
XtxP-12	82-0750	11	4	7	63.6
XtxP-19	204-1510	10	2	8	80.0
Total		59	17	42	71.0%

Table (9): Genotype specific-SSR marker in wheat.

Genotype	SSR markers	Total markers
Sahel -1	XtxP-19-473,497; XtxP-08-246,387	4
Giza -160	XtxP-12-397,750	2
Gemmeiza - 10	XtxP-10-364	1
Sids -1	XtxP-07-970	1
Beni-Sweif	XtxP-07-1620, XtxP-08-486	2
Sohag	XtxP-08-225, XtxP-12-479,50	3
Total		13

The RAPD-SSR based phonogram (Fig. 4) grouped the investigated genotypes into three main clusters. The first cluster included Sohag, the second cluster contain Sahel-1 and the third one was subdivided into two branches the first one has the cultivar Gemmeiza -10 the second one was subdivided into two branches the first one has Giza 160 the second one was then divided into three branches the first one has Sids-1, the second has Beni-Sweif and the third one was divided into two branches the first has Gemmeiza-9 and the second one contains Giza 168 and Gemmeiza -9.

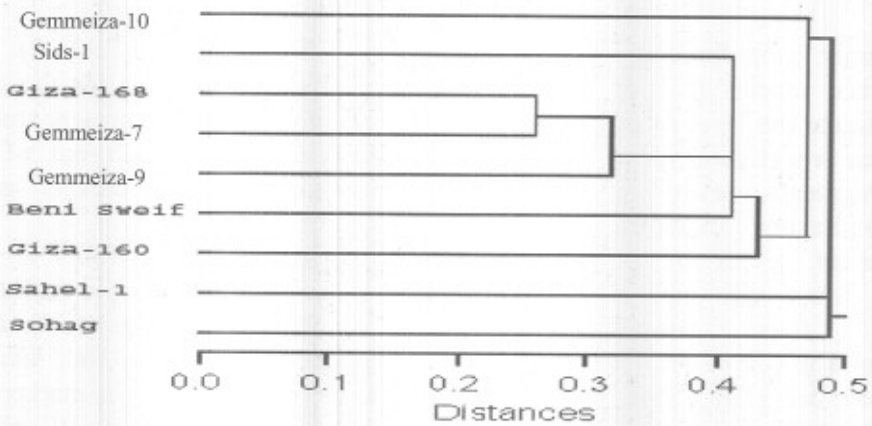

Fig.(4): Clustering of nine wheat genotype based on pooled RAPD and SSR markers.

DISCUSSION

Metabolic responses of higher plants to salt stress are complex, since many processes such as carbon metabolism, accumulation of compatible osmolytes, ion partitioning, energy metabolism and growth are modified (Cushman *et al.*, 1990). Some of the most dramatic responses to osmotic stress include osmotic adjustment and decreased cell expansion which may be associated with changes in cell wall properties (Binzel *et al.*, 1985). Salt stress decrease the concentrations of nitrogen, phosphorous, potassium and calcium as well as the K^+: Na^+ ratio, meanwhile the accumulation of Na^+ is increased in tissues of all the tested wheat genotypes. This result agreed with the findings reported by Sairam *et al.* (2002) and Ragab *et al.* (2008). The effects of Na ions in plants have bean well studied and it was found that Na is able to raise the pH of the soil, which in turn reduced the availability of P to the plant (Sonneveld and Voogt, 1983). Sodium was also found to displace membrane-bound Ca^{+2}(Shabala and Newman, 2000). A decrease in K^+ uptake may be due to a possible antagonism between K^+ and Na^+; this antagonism could be due to the direct competition between K^+ and Na^+ at the site of ion uptake at plasmalemma (Epstein and Rains, 1987). Na^+ could also enhance the efflux of K^+ into the growth medium, possibly due to membrane integrity (Shabala, 2000).It is well known that osmotic adjustment involves the net accumulation of solutes in a cell in response to salinity, and consequently, the osmotic potential decreases, which in turn attracts water into the cells and enables the turgor to be maintained (Neuman *et al.*, 1988). The wheat genotypes showed different responses to NaCl stress and the salt-tolerance in Gemmeiza 10, Sohag and Beni-Sweif are due to the higher degree of osmotic adjustment through the increasing in the uptake rate of K^+, Ca^{2+}, Pi and NO^{-3}, which greatly exceeded that in the salt susceptible one. With increasing salinity in the environment, the concentration of the protective solutes of different wheat cultivars was also increased. These results are in harmony with those obtained by Sairam *et al.* (2002), and Kafi *et al.* (2003). The total sugar concentrations were increased in response to salinity and this might be attributed to accelerated hydrolysis of starch and other polysaccharides and/or less

condensation of sugars to meet the increasing demand of osmotically active substances as well as the elevated respiration rate usually observed with salt stressed plants. The increment in the free amino acid levels can be partly explained by a generally increased proteolysis occurring during stress, and/or feedback regulation affected by the increased pools of free amino acids (Flores and Galston, 1984. Raising total free amino acids accumulation in salinized plants can act as components of salt tolerance mechanism and build up a favorable osmotic potential inside the cell in order to combat the effects of Cl which replaced nitrate in the vacuoles.

The salt stressed plants accumulate proline in their leaves. Sanada *et al.* (1995) demonstrated that proline has a bifunctional role in the accumulation to high salt stress; an osmoregulant role in the light to make water uptake easy, and a substrate for dark respiration to supply energy to compartmentation of ions into vacuole in the dark. So, it means that in response to osmotic challenge, the synthesis of compatible solutes such as sugars, total free amino acids and proline occurs as an indicator to the osmoprotectant levels in wheat plant and can be used as a biochemical marker for increased salt tolerance in this plant. Indeed, salt tolerance requires a net increase in the quantity of osmotically active solutes in the tissue. The tolerance of Gemmeiza 10, Sohag and Beni-Sweif genotypes was related to the accumulation of soluble sugars, free amino acids including proline, K^+ and Ca^{2+}. Isozyme loci have been used as markers in a number of genetic studies, such as genetic diversity in *Brassica juncea* (Kumar and Gupta, 1985, Persson *et al.*, 2001), and isozyme markers as seed coat color (Rahman, 2001).

Peroxidases are enzymes related to polymer synthesis in cell wall (Bowles, 1990), as well as it plays an important role in the prevention of oxidative damage caused by environmental stress to the membrane lipids (Kalir *et al.*, 1984). Salt stress increased peroxidase bands intensity. Salt tolerant cultivars Gemmeiza 10, Sohag and Beni-Sweif showed higher band intensity compared with the other cultivars. These results are in agreement with those of Gaspar *et al.* (1985) who reported an increase in peroxidase activity in cultivars sensitive to salt, which could be responsible for the ability of such cultivars to adapt to external stimulus.

In the present study, nine wheat genotypes were studied using SSR and RAPD markers. Since the PCR techniques have been developed a wealth of new DNA marker technologies has arisen enabling the generation of high-density molecular maps for all the major crop species. Molecular markers have also been extensively used to analyze the genetic diversity in crop plants. Based on the data obtained by RAPD analysis, it was possible to discriminate between the nine wheat genotypes used. The genotype-specific markers indicate that the highest number of RAPD specific markers was scored for Beni-Sweif (5 markers) while both of Giza-160, Gemmeiza -9 and Sohag scored three markers each. On the other hand, G-168 scored two markers and Sakha-1, Gemmeiza -10, Seds-1 scored one marker each. In the meantime, the highest number of RAPD genotype-specific markers was generated for primers OPN-13 (four markers). These markers can be verified as being RAPD markers associated with salt tolerance in the nine wheat genotypes.

SSR's were characterized in different eukaryotic organisms, being the most variable component of the genome with high rate of molecular evolution. The distribution and sequence of SSR markers may therefore, provide insight into phylogenetic relationships among varieties and species. The microsatllite variation is thought to be due to slippage of the

DNA polymerase during replication of unequal crossing over resulting in differences in the copy number of the core nucleotide sequence (Yu and Kohel, 1999; Qureshi *et al.* , 2004).In the present study all the SSR primers used produced amplifications with different levels of polymorphism, revealing 71% polymorphism. In total, 59 alleles were detected in the five SSR loci, with an average of 11.8 alleles per loci. The genotype specific SSR markers were determined, 13 markers can be considered as a useful marker for screening for salt tolerance in the nine wheat genotypes. The RAPD-SSR based dendrogram clustering the nine genotypes into different clusters reflecting their genetic relationships. Taking all the data together we can conclude that, the genotypes were ranked on the basis of the organic metabolites and inorganic ions accumulation. Based on the resalts, Gemmeiza 10, Sohag and Beni-Sweif genotypes were selected as tolerant and Giza 160 and Sids 1 genotypes as sensitive to salinity. The higher salt tolerance in these cultivars is due to their ability to maintain higher osmotic potential by accumulating much higher concentration of osmoticum solutes. The genotype specific molecular markers were determined and these markers can be considered as a useful marker for salt tolerance in wheat breeding programs.

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الملخص العربي

التباين الوراثى لصفة تحمل الملوحة بين بعض التراكيب الوراثية من اقمام الخبز و المكرونة

رضا علوانى مغيب*، عبد الهادى عبد الله عبد الهادى*، نيفين بهاءالدين طلعت**، سوسن سامى يوسف*، أحمد محمد الشرقاوى*
*قسم الوراثة – مركز بحوث ودراسات الهندسة الوراثية – كلية الزراعة – جامعة القاهرة
**قسم النبات الزراعى - فرع فسيولوجيا النبات - كلية الزراعة – جامعة القاهرة

لدراسة تأثير الخلفية الوراثية على تحمل القمح للملوحة تم تعريض بادرات سبعة اصناف من قمح الخبز *T. aestivum* و صنفين من قمح المكرونة *T.durum* لمعاملات ملحية لمدة اسبوعين. تم قياس كل من تحمل الضغوط الاسموزية ، السكريات الكلية و الاحماض الامينية الحرة و البرولين تحت ظروف الملوحة. اوضحت النتائج زيادة تركيز كل من الاحماض الامينية الحرة و البرولين بزيادة تركيز الملح، و قد اظهرت الاصناف جميزة 10 و سوهاج و بنى سويف اعلى تركيز للمركبات سالفة الذكر تحت ظروف الملوحة مقارنه بباقى الاصناف. أظهرت الاختلافات فى الضغط الاسموزى للاصناف تحت الدراسة علاقة ارتباط موجبه مع تركيز كل من ايونى الصوديوم و البوتاسيوم . و قد اتضح ان التحمل العالى للملوحة فى صنف جميزة 10 (*T. aestivum*) و لصنفى قمح المكرونة سوهاج و بنى سويف (*T. durum*) ترجع لقابلية هذه الاصناف للحفاظ على الضغوط الاسموزية للخلايا عند المستوى الذى يسمح بامتصاص المياه مقارنه بباقى الاصناف.كما اظهرت اصناف القمح جميزة 10 و سوهاج و بنى سويف كثافة عالية لشرائط البيروكسيديز تحت ظروف الملوحه مقارنة بباقى الاصناف. وكذلك تمت دراسة التباين الوراثى بين الاصناف المستخدمة باستخدام كل من تحليلى الRAPD و SSR و اظهرت نتائج ال RAPD ان عدد الواسمات الكلية المتحصل عليها كانت 118 من بينهم 82 واسم جزيئى متباين بنسبة 69.5% بينما اظهر ال SSR ان عدد الاليلات الكلية المتحصل عليها هو 95 من بينهم 42 اليل متباين بنسبة تباين 71%. و تعتبر هذه الواسمات مفيدة و يمكن استخدامها فى برامج التربية لاصناف القمح التسعة المستخدمة. وبالاضافه تم فى هذه الدراسة تحديد الواسمات الجزيئية المحددة لكل صنف و اتضح ان هناك سبعة واسمات جزيئية تميز الصنف جميزة 10 و يمكن اعتبار هذه الواسمات الوراثية متعلقة بصفة تحمل الملوحة فى الاصناف الثلاثة التى اظهرت استجابات عالية لتحمل الملح.