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*Full Length Research Paper*

# Molecular markers associated with salt tolerance in Egyptian wheats

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**Salinity affects plant growth by the osmotic stress of the salt around the roots, as well as by toxicity caused by excessive accumulation of salt in leaves. In the present study, seven common (*Triticum aestivum*) and two durum (*T. turgidum* ssp. *Durum*) wheat genotypes were subjected to salt stress for 2 weeks. 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 Gemmiza 10 and in *T. durum* cultivars Sohag and Beni Sweif was due to higher ability to maintain osmotic potential of the cells than the other cultivars by increase in osmoticum concentration under salt stress. The genetic variation and relationships among different wheat genotypes with different responses to salt stress were also investigated by RAPD and SSR analyses. 82 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 wheat cultivars tested. 18 random amplified polymorphic DNAs (RAPD) markers and 13 simple sequence repeats (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 Gemmiza 10. These markers can be verified as being genetic markers associated with salt tolerance in the three wheat genotypes and help in marker-assisted selection breeding program.**

**Key words:** 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 and water resources both in arid and semi-arid regions of the world (Ghassemi et al., 1995; Arzani, 2008). 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, while 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 salt tolerant genotype is a relatively difficult task due to the quantitative nature of salinity stress tolerance and the problems associated with developing appropriate and replicable testing environments (Arzani, 2008). 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).

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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'. Salt-tolerant cultivar may be considered as a substitute for amendments/reclamation on moderately saline soils and a supplement to amendments in strongly saline ones.

The random nature of the random amplified polymorphic DNAs (RAPDs) analysis (Williams et al., 1990) complements 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 Fournier, 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.

In addition, the simple sequence repeats (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). Secondly, SSRs can be analyzed by a rapid, technically simple and inexpensive polymerase chain reaction (PCR)-based assay that requires only small quantities of DNA. Thirdly, SSRs are co-dominant and simple Mendelian segregation has been observed. And 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.

The objectives of the present study were to investigate variation in nine wheat genotypes for their tolerance to salinity, to identify the physiological and biochemical processes that make a plant tolerant to salinity and 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 was conducted in the Genetic Engineering Research Center, Faculty of Agriculture- Cairo University, Egypt. In the present study, nine Egyptian wheat cultivars namely (Sahel-1, Giza-160, Giza-168, Gemmiza -7, Gemmiza -9, Gemmiza -10, seds-1, Benisouf 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 ≤ 60 kPa. At 30 days after 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 $\mu molem^{-2}s^{-1}$ (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 liquid nitrogen, and stored at -20°C. Tissues were thawed and centrifuged at 1,200 × g for 25 min at 4 °C to extract the cell sap. Osmotic potential ( $_s$) 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 ( $_s$) between salinized and control plants.

### Chemical analysis

Random samples of each treatment were used to determine the following chemical analysis. Nitrogen and phosphorus were determined according to Pregl (1945) and Jackson (1967), respectively. Potassium and sodium were determined using the flame photometer. 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) polyvinylpyrrolidone (PVP); 0.1% (w/v) ascorbic acid and 0.1% (w/v) cysteine) and centrifuged at 5333 × g (JS - 5.2 roter), at 4°C for 5 min. Twenty microliters 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 1 mM 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 1 h.

### Molecular analysis

### DNA extraction

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

### RAPD analysis

PCR reactions were conducted using arbitrary decamer primers (Operon Technology, Inc., Alameda, CA, USA). The names and

**Table 1.** Names and sequences of the primers used for RAPD analysis.

| 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 |

**Table 2.** Types and sequences of the SSR loci and annealing temperature for PCR reaction.

| Locus | Type of SSR (s) | Sequence of forward primers | Sequence of reveres primers | Annealing temperature |
|---|---|---|---|---|
| ***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*** |

sequences of the primers that give clear bands are listed in Table 1. The reaction mixture (20 µL) contained 10 ng DNA, 200 M dNTPs, 1 µM primer, 0.5 U of Red Hot Taq polymerase (AB-gene House, UK) and 10-X Taq polymerase buffer (AB-gene House, 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 ethidium 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 pair-wise comparison for the proportion of shared bands amplified (Nei 1987).The similarity coefficients was 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 in Table 2.

#### Polymerase chain reaction (PCR)

The polymerase chain reaction was carried out in a Biometra thermal cycler using primers listed in Table 2. The PCR reaction mix includes the following: DNA, 10 ng/µL; 0.5 U of Red Hot Taq polymerase (AB-gene House, UK) and 10-X Taq polymerase buffer (AB-gene House, UK); 10 mM dNTPs; 50 mM $MgCl_2$; 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 ethidium 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). 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 3). The osmotic potential ( $_s$) of the salt treated plants decreased with increasing NaCl concentration and the decrease was more pronounced in Gmiza-10. Osmotic adjustment (OA) increased with NaCl concentration, and was greater in Gmiza-10 followed by

**Table 3.** Osmotic potential ( $_s$) and osmotic adjustment (O.A.) in nine wheat cultivars under salinity stress.

| Parameter | NaCl (mM) | Wheat genotype | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Sahel-1 | Giza 160 | Giza 168 | Gemmiza 7 | Gemmiza 9 | Gemmiza 10 | Seds-1 | Beni-Sweif | Sohag |
| $_s$ (MPa) | 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** |

**Table 4.** Nitrogen, phosphorus and calcium concentration (mg $g^{-1}$ DW) in the nine wheat cultivars under salinity stress.

| Parameter | NaCl (mM) | Wheat genotype | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Sahel-1 | Giza 160 | Giza 168 | Gemmiza 7 | Gemmiza 9 | Gemmiza 10 | Seds-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 |

Sohag then Beni- Sweif and lowest in Gmiza-7. The difference in $_s$ 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

Furthermore, the concentration of total sugars, total free amino acids and free proline of different wheat genotypes was increased by salinity. Gmiza 10, Sohag and Beni-Sweif genotypes accumulated the highest concentrations while Giza 160 and Seds-1 maintained the lowest concentrations followed by Sahel 1, Gemmiza 9, Gemmiza 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. The maximum ratios and concentrations were observed in Gemmiza 10, Sohag and Beni-Sweif genotypes and the minimum were observed in the case of Giza 160 and Seds-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. Gemmiza 10 and Sohag genotypes proved to be the best of all; they maintained a higher accumulation of organic metabolites and inorganic ions (Tables 4 to 6). Moreover, salinity stress increased band intensity of the salt treated wheat plants much higher than the control plants in all cultivars. The increase in peroxidase level was with increasing salt concentration. The level of band intensities, however, differed between cultivars (Figure 1).

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. In this study, a total of 118 DNA markers were detected among the nine

**Table 5.** $Na^+$ and $K^+$ concentration (mg $g^{-1}$ DW) in the nine wheat cultivars under salinity stress.

| Parameter | NaCl (mM) | Wheat genotype | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Sahel-1 | Giza 160 | Giza 168 | Gemmiza 7 | Gemmiza 9 | Gemmiza 10 | Seds-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 6.** The effect of salinity stress on total sugar, free amino acids and proline concentration in nine wheat cultivars.

| Parameter | NaCl (mM) | Wheat genotype | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Sahel-1 | Giza 160 | Giza 168 | Gemmiza 7 | Gemmiza 9 | Gemmiza 10 | Seds-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 |

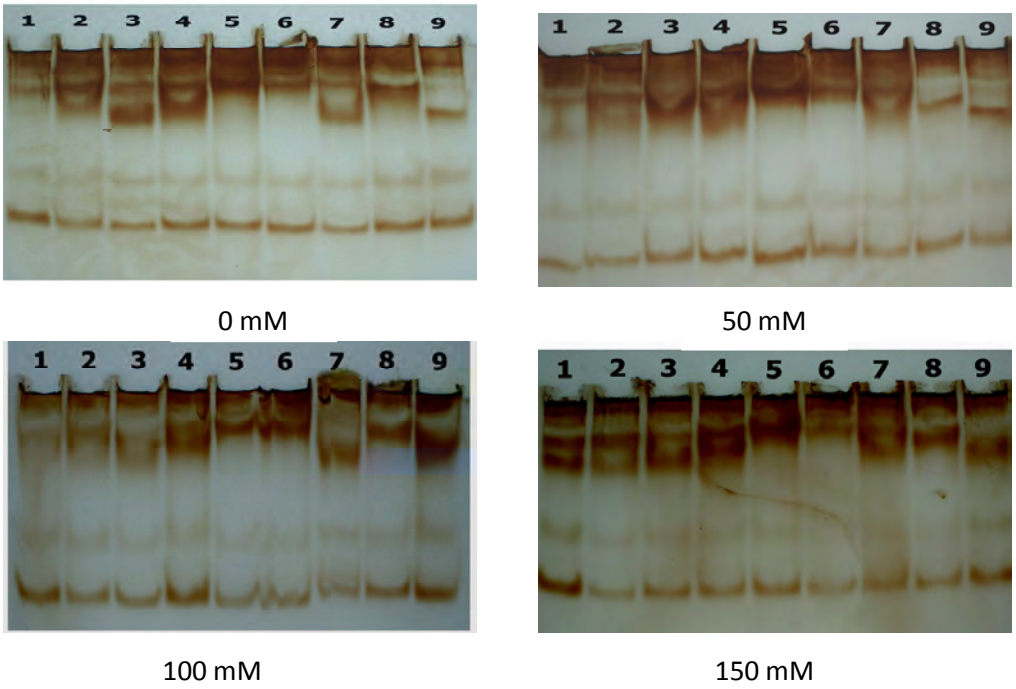


**Figure 1.** Peroxidase isozyme profile of the control and salt treated wheat plants under salinity stress. Lanes 1-9: the wheat cultivars Sahel, Giza 160, Giza 168, Gmiza7, Gemmiza 9, Gemmiza 10, Seds 1, Beni Sweif and Sohag , respectively.

**Table 7.** Primers used in RAPD analysis and their number of bands.

| Primer name | Number of scorable bands | Polymorphic bands | Percentage 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 |

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 (Figure 2 and Table 7 ). 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). The genotype specific RAPD markers for the different wheat cultivars used in the present study are listed in Table 8. 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 Giza-160, Gemmiza -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).

In addition, 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).

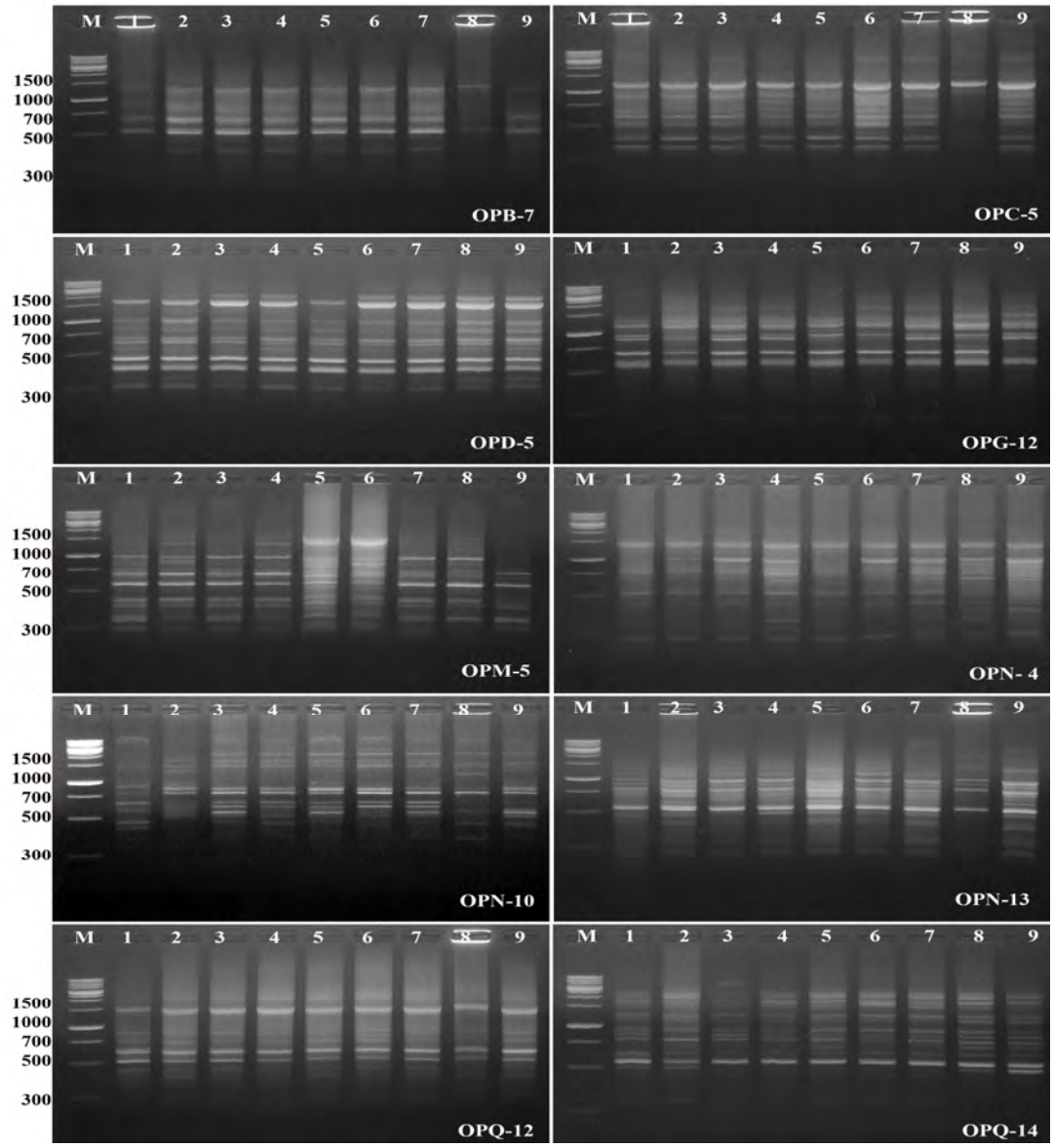


**Figure 2.** RAPD banding patterns of nine different wheat genotypes using ten selected random primers, M: 1 kbp plus DNA ladder; Lanes 1 to 9, the wheat cultivars Sahel, Giza 160, Giza 168, Gemmiza 7, Gemmiza 9, Gemmiza 10, Seds-1, Beni- Sweif and Sohag, respectively.

**Table 8.** Genotype – specific RAPD – markers.

| Genotype | Marker | 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 |
| Gemmiza -7 | - | - |
| Gemmiza -9 | OPM-05 (373), OP N-13 (388), OPN-04 (1055) | 3 |
| Gemmiza - 10 | OPO-12 (780) | 1 |
| Seds-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 |

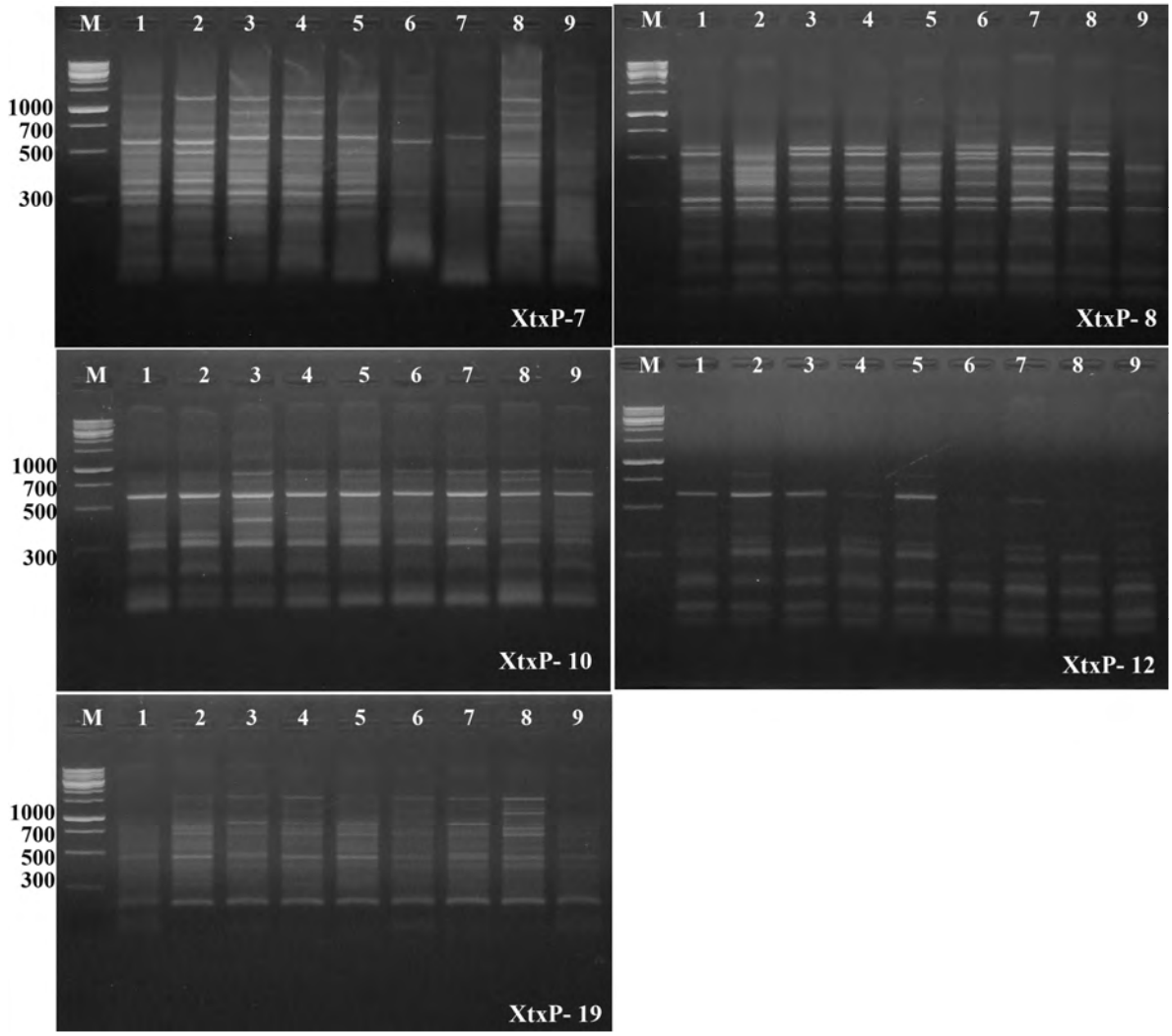


**Figure 3.** SSR banding patterns of nine different wheat genotypes using five primers, M: 1 kbp plus DNA ladder; lanes 1 to 9, the wheat cultivars Sahel, Giza 160, Giza 168, Gmiza7, Gemmiza 9, Gemmiza 10, Seds-1, Beni-Sweif and Sohag, respectively.

Most of the alleles were polymorphic, thus revealing 71% polymorphism. The size of the detected alleles producedfrom using the SSR primer sets ranged from 82 to 1620 bp, which reflects a large difference in the number of repeats between the different alleles (Table 9). The genotype-specific SSR markers for the different wheat cultivars used in the present study are listed in Table 10. Thirteen out of the forty-two polymorphic SSR markers generated were found to 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 both of Giza-160; Gemmiza -9 and Beni-Sweif scored two markers each. On the other hand, Gemmiza -10 and Seds-1 scored one marker each.

The RAPD-SSR based phonogram (Figure 4) grouped the investigated genotypes into three main clusters. The first cluster included Sohag, the second cluster contained Sahel-1 and the third one was subdivided into two branches; the first one have the cultivar Gemmiza -10, the second one was further subdivided into two branches; the first one has Giza 160 and then the second divided into three branches the first one Seds-1, the second Beni-Sweif and the third one divided into two more branches; the first Gemmiza -9 and the second one contained Giza 168 and Gemmiza -9.

## DISCUSSION

Metabolic responses of higher plants to salt stress are complex, since many processes such as carbon metabolism, accumulation of compatible osmolytes, ionpartitioning, energy metabolism and growth are modified (Cushman et al., 1990; Arzani, 2008). Some of the most dramatic responses to osmotic stress include osmotic adjustment and decreased cell expansion which

**Table 9.** Number of alleles, fragment size range and polymorphism detected by SSR loci in the nine wheat genotypes.

| Primer | Fragment size (bp) | Numberof alleles | Monomorphic bands | Polymorphic bands | Polymorphic (%) |
|---|---|---|---|---|---|
| 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 | 082-0750 | 11 | 4 | 7 | 63.6 |
| XtxP-19 | 204-1510 | 10 | 2 | 8 | 80 |
| Total | | 59 | 17 | 42 | 71 |

**Table 10.** Genotype specific-SSR marker in wheat.

| Genotype | SSR marker | Total markers |
|---|---|---|
| Sahel -1 | XtxP-19-473,497; XtxP-08-246,387 | 4 |
| Giza -160 | XtxP-12-397,750 | 2 |
| Gemmiza - 10 | XtxP-10-364 | 1 |
| Seds-1 | XtxP-07-970 | 1 |
| Beni Sweif | XtxP-07-1620, XtxP-08-486 | 2 |
| Sohag | XtxP-08-225, XtxP-12-479,508 | 3 |
| Total | | 13 |

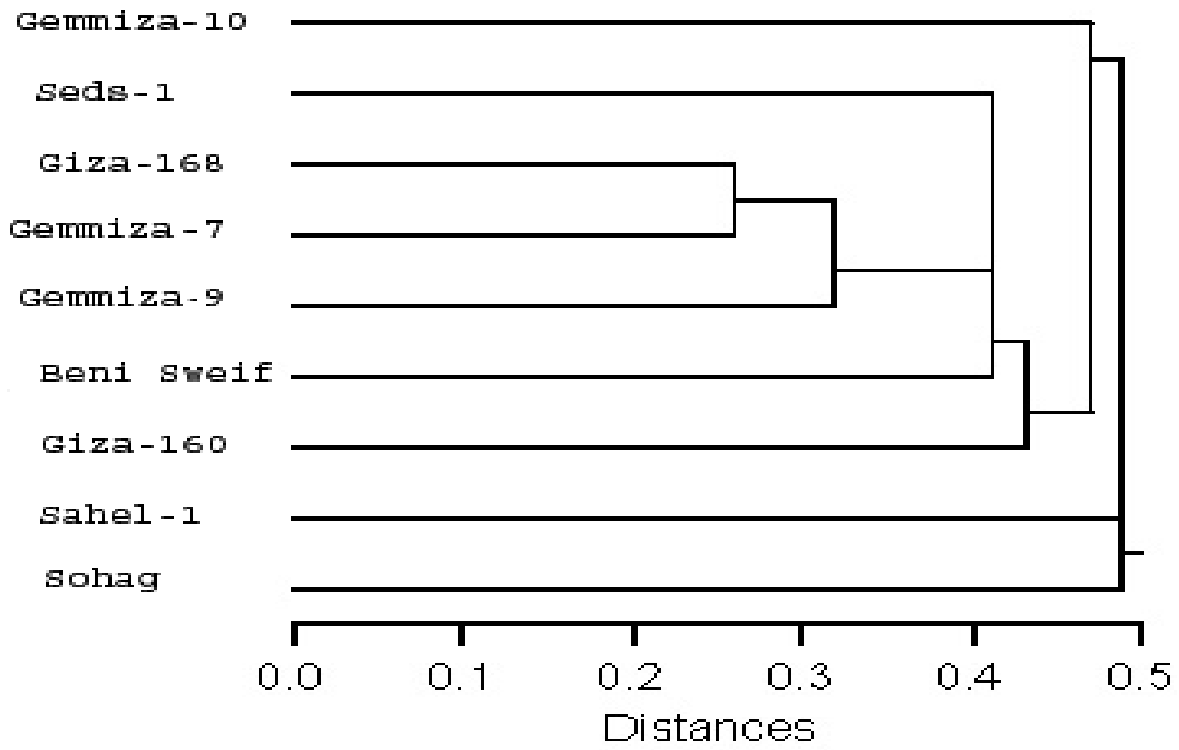


**Figure 4.** Clustering of nine wheat genotype based on pooled RAPD and SSR markers.

may be associated with changes in cell wall properties (Binzel et al., 1985). Salt stress decreases 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) in bread wheat and Houshmand et al. (2005) in

durum wheat. The effects of Na ions in plants have been well studied and it was found that Na is able to raise the pH of the soil, which in turn reduces 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 Gemmiza 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).

It was also observed that the total sugar concentrations 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. In addition, the salt stressed plants accumulated proline in their leaves. Sanada et al. (1995) demonstrated that proline has a bifunctional role in the accumulation to high salt stress; an osmoregulator 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. Hence, 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 Gemmiza 10, Sohag and Beni-Sweif genotypes was related to the accumulation of soluble sugars and 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 in the prevention of oxidative damage caused by environmental stress to the membrane lipids (Kalir et al., 1984). It was found that salt stress increased peroxidase bands intensity. Salt tolerant cultivars Gemmiza 10, Sohag and Beni-Sweif showed higher band intensity compared with the other cultivars. These results were 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 indicated that the highest number of RAPD specific markers was scored for Beni-Sweif (5 markers), while both of Giza-160, Gemmiza -9 and Sohag scored three markers each. On the other hand, G-168 scored two marker 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). These markers can be verified as being RAPD markers associated with salt tolerance in the nine wheat genotypes.

More also, SSR 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 microsatellite 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.

## Conclusion

Ranking genotypes based on organic metabolites and inorganic ions accumulation, it could be suggested that Gemmiza 10, Sohag and Beni-Sweif are salt tolerant, while Giza 160 and Seds-1 are salt sensitive. The higher salt tolerance in these cultivars was due to their ability to maintain higher osmotic potential by accumulating much higher concentration of osmoticum solutes. According to the genotype specific molecular markers determined, these markers can be considered useful for salt tolerance in wheat breeding programs.

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