EVALUATION OF YIELD AND SALT TOLERANCE OF TWO BARLEY CULTIVARS IN THREE LOCATIONS OF DIFFERENT SALINITY LEVELS

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ABSTRACT

This study was conducted over two seasons 2011/12 and 2012/13 to evaluate yield and salt tolerance of two barley cultivars cultivated in three locations of different salinity levels. The three locations were at El Qantra Shark and Sahel El Tinna regions which differed in their salinity levels (1.2, 13.2 and 20.5 ds m^{-1}). Two barley cultivars were Giza 125 and 132. Some morphological, physiological and productivity parameters were evaluated at 105 days after sowing (DAS) and at harvest. Estimation of plant height, flag leaf blade area, total chlorophylls, relative water content, leaf osmotic potential, proline, K$^+$, Na$^+$ contents, number of spikes m^{-2}, 1000 grain weight and grain yield indicated that Giza 125 cv. was more tolerant to salinity compared to Giza 132 cv. In general, salinity tolerance was contributed with high levels of total chlorophylls, relative water content, leaf osmotic potential, proline and K$^+$ contents. Although Na$^+$ was accumulated to high levels (55mg g^{-1}DW) in leaves at 105 DAS but it was decreased (150 µg g^{-1} DW) in grains at harvest. About 23-29 of protein bands were detected in Giza 125 cv. compared to only 20 bands in Giza 132 cv. Detection of unique bands 67 and 34kDa at the high level of salinity (20.5 ds m^{-1}) in Giza 125 cv. could be used as a molecular marker for selection of salt-tolerant genotypes in barley. However, Giza 132 cv. expressed 9-11 bands of isoesterases compared to only 7 bands in Giza 125 cv. under salinity stress. On the other hand, 6 isobands of peroxidases were expressed in Giza 125 cv. compared to only 5 bands in Giza 132 cv. under high salinity conditions. It could be concluded that, unique bands of protein or isoesterases are useful biomarker for selecting salt tolerant genotypes in barley which contributed with high yield potential, as was expressed in more number of spikes m^{-2} and as well high number of heavier 1000-grain weight and finally in the straw and grain yields/fad.

Key words: Salinity, barley, productivity, osmolytes, protein electrophoresis, isoesterase, isoperoxidase.

INTRODUCTION

High salinity is a common abiotic stress factor that seriously affects crop production in some parts of the world, particularly in arid and semi-arid regions. Increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within the next 25 years and up to 50% by the middle of 21st century (Wang et al., 2003). Salt stress causes many adverse effects on growth, development, yield and its quality of the harvested products of the cultivated plants

(Ashraf and Harris, 2004), due to a high osmotic potential of soil solution (osmotic stress), specific ion effects (ion stress) and production of reactive oxygen species (oxidative stress), (Flowers et al., 1977; Greenway and Munns, 1980).

Biochemical strategies of plant salt tolerance though a number of osmotic adjustment process include, selective accumulation or exclusion of ions, control of ion uptake by roots and transport into leaves, compartmentalization of ions at the cellular and whole-plant levels, synthesis of compatible solutes, change in photosynthetic

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pathway, alteration in membrane structure, induction of antioxidative enzymes, and induction of plant hormones as reviewed by Parida and Das (2005).

Ion uptake and compartmentalization are crucial not only for normal growth but also for growth under saline conditions (Adams *et al.*, 1992) because the stress disturbs ion homeostasis. However, salt-tolerant plants maintained high concentrations of K^+ and low concentrations of Na^+ in the cytosol to avoid their deleterious effect on functional macromolecules in the cell (Zhu *et al.*, 1993). Also, inhibition of Na^+ transporters on plasma membrane prevents Na^+ influx to plant cell cytosol (Zhu *et al.*, 1993). Quenching of Na^+ ions from the cytosol or compartmentalization it in the vacuoles is done by a salt-inducible Na^+/H^+ antiporters (Apse *et al.*, 1999).

Cytosol of salt-stressed plants avoids desiccation by accumulation of compatible solutes or osmolytes as glycine betaine, proline, and polyols (Shabala and Cuin, 2006) to increase the osmotic potential or to protect the important enzymes in the cell (Bray, 1993). Water deficit due to salinity leads to the formation of reactive oxygen species (ROS), which seriously disrupt normal metabolism through oxidative damage of lipids, proteins, enzymes, pigments and nucleic acids (Fridovich, 1986). Plants possess a number of antioxidants that protect against the potentially cytotoxic species of activated oxygen. High level of non-enzymatic antioxidants as polyphenols, carotenoids, ascorbic acid and proline was determined in many tolerated cultivars of crops. The lower activity of enzymatic antioxidant as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) was detected in other sensitive genotypes of crops. SOD scavenged superoxide (O_2^-) but POD and CAT catalyze the breakdown of H_2O_2 to H_2O and O_2 (Chang *et al.*, 1984). Genetic modified crops with high levels of antioxidants capacity or over expression of compatible solutes showed high tolerance to salinity (Hayashi and Murata, 1998).

Barley is used as forages and as human food because of its high nutritional and biological values. It is considered a highly salt-tolerant compared to other Triticeae members, where their tolerance is genotypic depended. Salinity threshold of barley is about 8 ds m^{-1} and every increment of salt unit (1ds m^{-1}) reduced their yield by about 5% (Maas and Hoffman, 1977).

Electrophoretic techniques for proteins and isoenzymes polymorphism have been used as an identification and quantification methods which provide correlation between the altered expression of specific genes and changes in the environment. These changes in expression of genes would be involved in adaptation and could be used as molecular markers for salt stress. One-dimensional polyacrylamide gel electrophoresis of proteins has been used extensively for identification and classification at the strain and species level. Also, isoenzymes have been widely used to screen the variability present among population and to select the desirable genotypes (Gong *et al.*, 2005).

Esterases (EST: EC, 3.1.1.1), a group of hydrolases, catalyze the cleavage of ester bonds of important compounds in plants as triglyceides of membranes, chlorophylls, essential oils and DNA. The relationship between esterase activity and salinity has been investigated in several plant species, irrespective of their tolerance to salt (Hassanein, 1999).

Plant peroxidases (POD: EC, 1.11.1.7) are heme-containing enzymes whose primary function is to oxidize a variety of hydrogen donors at the expense of hydrogen peroxide. Peroxidase activity has been correlated with a wide range of plant physiological processes, including lignification, suberization, somatic embryogenesis, auxin metabolism, wounding, and disease resistance (Ye *et al.*, 1990; Zimmerlin *et al.*, 1994). PODs are ubiquitous enzymes in plants, often occurring as multiple isoforms; for example, they are encoded by 73 different genes in *Arabidopsis thaliana* (Duroux and Welinder, 2003). Such an abundance of isoforms is consistent with diverse physiological functions for the peroxidase family (Siegel, 1993). POD polymorphisms are often used in taxonomic and population studies.

Although the mechanisms utilized by plants to survive under saline conditions is well understood but there are no well-defined indicators for salinity tolerance available to assist plant breeders to select new salt-tolerant genotypes for important agricultural crops (Ashraf and Harris, 2004). Therefore,

comparative analysis between two cultivars of barley in salt tolerance experiment during two seasons was conducted to evaluate their productivity and investigate some molecular and physiological traits which contribute to their tolerance to salinity.

MATERIALS AND METHODS

Experimental Sites and Conditions

Experiments of surface irrigation system were performed in El-Qantara Shark and Sahel El-Tinna regions at Ismailia and Port Said Governorates during 2011/12 and 2012/13 in three sites differed in their salt levels. The experiments aimed to test the salt tolerance of two six-rowed barley cultivars (Giza 125 and Giza 132) and to evaluate their productivity. Prior to the commence of the experiments, soil samples from each site were obtained with an auger from soil depths of 0-60 cm to determine some soil physical and chemical properties of the experimental sites (Table 1a). A mechanical soil analysis was carried out using the pipette method (Black, 1973). The soil reaction (pH) was measured with Backman pH meter, Electrical conductivity and soluble cations and anions were determined in the soil-paste extract (Page et al., 1982). Soluble Na^+ and K^+ were determined by a flame photometer, whereas soluble Ca^{2+} and Mg^{2+} were determined using the versenate method according to Richards (1954). Cl^-, CO_3^{-2} and HCO_3^- were determined by titration against silver nitrate for chloride and HCl for the other two anions, as described by Jackson (1973). The sulphate was calculated by the differences between cations and anions. Cation exchange capacity (CEC) and exchangeable cations were determined using ammonium acetate method (Jackson, 1973). Sodium adsorption ratio (SAR) and Exchangeable sodium percentage (ESP) were calculated according to Page et al., 1982. Osmotic potential (MPa) was calculated by multiplied EC x 0.036 (Khaliq et al., 2012).

The three sites were irrigated by mixed water (Nile + sewage in 1:1 ratio) which was obtained from Al-Salam Canal. Before the start of the experiment, water samples from Al-Salam Canal were taken for determination of pH, EC, soluble cations (Na^+, Mg^{2+}, Ca^{2+} and K^+), soluble anions (Cl^-, $HCO3^-$, $SO4^{-2}$) according to the method described by Hess, 1963 (Table 1b). Also, during the two growing seasons, the monthly electrical conductivity (EC, ds/m) of the three experimental locations and the irrigation water were determined (Table 2).

Experimental Design, Treatments and Agronomic Practices

A field experiment was designed in a randomized complete block design with 4 replicates, in each site of different salinity level and was analyzed separately. A combined analysis was performed over locations to get the effect of salinity and their interaction. A homogeneity test of experimental error for locations was calculated before analysis. Each site was occupied by two barley cultivars (Giza 125 and Giza 132). The first site expresses the normal salinity level (control, EC= 1.25 ds m^{-1}). The second site had EC of 13.2 ds m^{-1} and the third had EC of 20.5 ds m^{-1}. The plot area was 10.5m² (3 x 3.5) including 15 rows, 20 cm apart. Barley grains (Hordeum vulgare L.) were sown on 8th of December in the first and second seasons at a seeding rate of 50kg/fad. Nitrogen fertilizer was applied at a level of 65 kg N/fad., as ammonium sulphate (20.5% N) in four equal doses, beginning with the first irrigation until heading. Phosphorus fertilizer was applied at a level of 31 kg P_2O_5/fad., as calcium superphosphate (15.5% P_2O_5). Potassium fertilizer was applied at a level of 24 kg K_2O/fad., as potassium sulphate (48% K_2O). Phosphorus and potassium fertilizers were applied before sowing in all treatments. The other agronomic practices were done as recommended.

Sampling

At 105 days from sowing (DFS), barley plants from an area of 0.5 m² in each plot were randomly taken for determining plant height, flag leaf blade area, relative water content (RWC), leaf osmotic potential, prolinc, total chlorophylls and leaf Na^+ and K^+ contents, SDS-PAGE of protein, PAGE of isoperoxidase and isoestrase. All these parameters were determined in flag leaf in both seasons except SDS-PAGE of protein and PAGE isoenzymes in second seasons. At harvest, an area of 2m² from each plot was harvested to determine yield, its components and grain Na^+ and K^+ contents.

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Table 1a. Soil physical and chemical properties of the experimental sites at sowing for the upper 60 cm soil depth (averaged over 2011/12 and 2012/13 seasons)

| Locations | EC ds/m | pH | mmol l^{-1} | | | | | | |
			Ca^{2+}	Mg^{2+}	Na^+	K^+	HCO_3^-	Cl^-	SO_4^{-2}
L1	1.2	7.1	3.1	1.6	7.8	0.5	2.3	8.5	2.2
L2	13.2	8.2	13.5	58.3	102.3	5.1	12.5	155.2	12.5
L3	20.5	8.2	18.2	91.5	153.0	5.4	4.2	234.2	21.0

Locations	EC ds/m	Sand (%)	Silt (%)	Clay (%)	Texture class	Exchangeable Na^+ (cmol kg^{-1})	CEC (cmol kg^{-1})	SAR	ESP (%)	Ψs (-Mpa)
L1	1.2	92.7	3.1	4.2	sand	0.32	5.4	5.1	5.9	0.0432
L2	13.2	78.5	15.3	6.2	sand	2.2	5.9	17.1	37.3	0.4752
L3	20.5	75.2	15.7	9.1	sand	1.80	5.5	20.7	32.7	0.7380

SAR, sodium absorption ratio, ESP, Exchangeable sodium percentage, CEC, Cation exchange capacity, L1, Location1, L2, Location2, L3, Location3.

Table 1b. Water chemical properties of Al-Salam Canal (Nile + sewage water, 1:1) at sowing (averaged over the two seasons).

| EC ds/m | pH | mmol l^{-1} | | | | | | | |
		Ca^{2+}	Mg^{2+}	Na^+	K^+	HCO_3^-	Cl^-	SO_4^{-2}	SAR
1.90	7.99	3.8	2.5	13.4	0.35	3.5	3.2	3.4	7.5

SAR, sodium absorption ratio

Table 2. Monthly electrical conductivity (EC, ds/m) of the three experimental locations, L, and the irrigation water, IR, at sowing and during the growing durations of barley in 2011/12 and 2012/13 seasons

| | 2011/12 (EC, ds/m) | | | | | | | 2012/13 (EC, ds/m) | | | | | | |
	Dec.	Jan.	Fab.	Mar.	Apr.	May.	Aver.	Dec.	Jan.	Fab.	Mar.	Apr.	May.	Aver.
L1	1.10	1.10	1.20	1.20	1.40	1.40	1.23	1.30	1.20	0.98	1.10	1.20	1.20	1.16
L2	13.0	13.1	13.0	13.5	13.6	13.6	13.30	13.4	13.4	13.1	12.9	13.2	13.2	13.20
L3	20.6	20.2	20.4	20.5	20.9	20.9	20.58	20.4	20.4	20.1	20.2	20.2	20.7	20.33
IR	1.89	1.85	1.85	1.95	2.00	1.99	1.92	1.93	1.83	1.90	1.88	1.90	1.92	1.89

IR, (Nile + sewage water, 1:1)

Plant height

Plant height (cm) was determined from the soil surface to the top of the main spike.

Flag leaf blade area (LA)

LA was determined by scanning the flag leaf with a leaf area meter (AM 300).

Chlorophyll assay

According to Arnon (1949), 0.5 g fresh flag leaves was ground with 10ml aceton 85% and filtered. Optical density was measured at 644 and 662 nm using a Beckman DK-2 Spectrophotometer. Concentration of total chlorophylls as mg/100 g FW was calculated.

Relative water content

Relative water content was estimated using the following formula: RWC= (FW-DW)/ (TW-DW) x100, where FW is the average weight of freshly twenty leaf disks collected from flag leaves, TW the weight of disks after hydration for 12hr at room temperature under low light conditions and DW is the average weight of the same disks after drying at $80C^0$ for 48hr, according to Henson et al. (1981).

Leaf osmotic potential

Osmotic potential (Ψs) in -Mpa was determined with constant weight method using serial sucrose solutions according to Moore (1974).

Leaf proline content

Free proline was assayed in fresh flag leaf according to Bates et al. (1973). L-proline was used as a standard.

Flag leaf Na^+ and K^+ content

Random sample of flag leaves from each plot was dry-ashed at 550°C for 4hr, then 0.5 g of powdered material was digested using a mixture of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) and then brought to a final volume of 50 mL with distilled water. The content of potassium and sodium was determined by flame photometer model III (Carl Zeiss Jena, Germany) according to Brown and Lilleland (1946).

SDS-PAGE of protein

Flag Leaves blades (0.5 g) were ground in a mortar and defatted twice with 70% (v/v) ethanol (50ml/g) for 10 min at 4°C. Total soluble proteins were extracted with extraction buffer (0.1g/ml) containing, 20mM Na-borate buffer, 0.5 M NaCl, 1 mM of ethylendiaminetetra acetic acid (EDTA), pH 8.9. After 12 hours of stirring the extract was centrifugated at 10.000 r.p.m./min. for 20 min. The supernatant (10 µl) was taken for electrophoresis according to Matta et al. (1981) and Tucci et al. (1991). SDS-PAGE was carried out in 10% acrylamide slab gels, with a current of 25 mA and 130 V per gel until the bromophenol blue marker reached to the bottom after 3 hours, according to Laemmli (1970). After electrophoresis the gel was stained using silver staining as described by Blum et al., (1987). After staining, the slab gel was washed to remove the excess of staining solution in acetic acid (7%) and distilled water. Then the gel was photographed and made by scan apparatus. Moreover, similarity % (= number of similar bands / total number of bands x 100) of both positive and negative data was calculated according to Ladizinsky and Waines (1982).

PAGE- isoenzymes

The isoenzymes extracts were prepared by crushing small pieces cut from green leaves (0.5g) in 0.2 ml of a cold buffer, containing 0.05 M Tris- hydroxymethyl aminomethan, 0.01 M of (EDTA) and 5mM dithiotheritol in a mortar under cooling. The mixture of each was centrifuged at 6.000r.p.m./15 min. at 4°C, then 100µl of 50% (v/v) glycerol and 0.05 mg/ml bromophenol blue (tracking day) were added. Then 20 µl of each sample was loaded for electrophoresis (Jasska and Jasska, 1988). Gel electrophoresis was carried out according to the following condition: Acrylamide concentration 8%, dissolved in Tris-HCl, running buffer composed of 0.005 M Tris + 0.038M glycine, adjust pH to 8.3 with 1M Tris solution, running time (2 hours) and running temperature (4°C) according to Steven and Thomas (1986). All stained gels were fixed in a mixture containing H_2O: ethanol : acetic acid: glycerol (2:1:1:1). The stained isoenzyme patterns were scanned densitometrically.

Esterase (carboxylesterase) isoenzymes visualization (EC 3.1.1.1)

The esterase isoenzymes were visualized according to modified procedure described by Balen et al. (2003). 2-naphthyl acetates, used as

substrates (40 mg each), were dissolved in 16 mL of 50% (v/v) acetone and mixed with 100 mL of 50mMTris/HCl pH 7.1. After staining (30 min), the gels were washed with tap water and incubated in a solution containing 50 mM Tris/HCl pH 7.1 and Fast Blue RR salt until purple red bands appeared (20–30 min). The Fast Blue RR salt (200 mg) was dissolved in 10 mL of absolute methanol and filtered into 50 mM Tris/HCl pH 7.1. The gels were once more rinsed with tap water and fixed in 30% (v/v) ethanol.

Peroxidase isoenzymes visualization (EC 1.11.1.7)

Peroxidase isoenzymes were detected by incubating the gels for 5–20 min in a reaction mixture containing 0.5 mM benzidine hydrochloride and 10 mM H_2O_2 in 0.05 M acetate buffer, pH 4.9 according to the procedure of Ornstein (1964).

Yield Measurements

At harvest, 1000-grain weight (g), number of spikes m^{-2}, grain yield (ardab fad^{-1}) and straw yield (ton fad^{-1}) were determined.

Grain Na^+ and K^+ contents

Random grain sample from each plot was dry-ashed at 550°C for 4hr, then 0.5g of powdered material was digested using a mixture of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) and then brought to a final volume of 50 mL with distilled water. The content of potassium and sodium was determined by flame photometer model III (Carl Zeiss Jena, Germany) according to Brown and Lilleland (1946).

Statistical Analysis

The analysis of variance of randomized complete block design was used according to Snedecor and Cochran (1982). The combined analysis of variance was performed for the data of the two seasons and locations after test the homogeneity of error by Bartellet test (Steel *et al.*, 1997). Averages followed by the same alphabetical letters are not statistically different according to Duncan's Multiple Range Test at the 5% level of significance (Duncan, 1955).

RESULTS

The results recorded in Tables 3, 4, 5 and 6 revealed that there were remarkable decreases in all measured parameters by increasing the salinity level, except for the Na^+ content in flag leaf and grain as well as proline which were increased. The same trend was observed in Giza132 cv., except for level of Na^+ in flag leaf and grain as it was increased compared with Giza 125 and that held true in both seasons as well as over them. The combined analysis of variance for the data over the two seasons and locations reveled that, there were significant interaction effects between salinity levels and cultivars on all parameters except plant height and straw yield (Tables 3, 4, 5 and 6). The results and discussion were focused on the interaction effect of salinity levels and cultivars on all measured parameters as follows:

Flag Leaf Blade Area (LA)

Results cited in Table 7 showed that the two cultivars (Giza 125 and 132) recorded a significant maximum values of flag leaf blade area (about 11.87 and 12.87 cm^2, respectively) under the low level of salinity S1 (1.2 ds/m) without significant differences between them. On contrary, increment of salt levels decreased LA by about 27 and 42% in leaves of Giza 125 cv. and by 55 and 66% in Giza 132 cv., under the 13.2 and 20.5 ds/m, respectively compared to control (S1) with significant differences between them. Under salt stress, Giza 125 cv. significantly surpassed Giza 132 cv. in LA, e.g., Giza 125 under high level of salinity (S3) gave LA (6.92cm^2) higher than Giza 132 (5.78 cm^2) under moderate level of salinity (S2).

Total Chlorophylls

Under normal salinity level (S1), the leaves of the two cultivars synthesized maximum amount of total chlorophyll (29.6 and 30.4 mg $100g^{-1}$ in Giza 125 and 132, respectively), without significant differences between them. Degradation of chlorophyll in leaves of Giza 132 cv. (61 to 72%) was significantly higher than Giza 125 cv. (33 to 49%) which was subjected to S2 and S3 levels of salinity, respectively (Table 7). It is clear that chlorophyll molecules in Giza 125 cv. had vigor protection mechanism than in Giza 132 cv. under high level

substrates (40 mg each), were dissolved in 16 mL of 50% (v/v) acetone and mixed with 100 mL of 50mMTris/HCl pH 7.1. After staining (30 min), the gels were washed with tap water and incubated in a solution containing 50 mM Tris/HCl pH 7.1 and Fast Blue RR salt until purple red bands appeared (20–30 min). The Fast Blue RR salt (200 mg) was dissolved in 10 mL of absolute methanol and filtered into 50 mM Tris/HCl pH 7.1. The gels were once more rinsed with tap water and fixed in 30% (v/v) ethanol.

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Table 3. Effect of salinity levels and cultivars on plant height, flag leaf blade area, total chlorophyll and relative water content in leaves of barley plants at 105 days from sowing in the two seasons and their combined

Factors	Plant height (cm)			Flag leaf blade area (cm^2)			Total chlorophyll (mg 100g^{-1} FW)			Relative water content (%)		
	2011/12	2012/13	Comb.	2011/12	2012/13	Comb.	2011/12	2012/13	Comb.	2011/12	2012/13	Comb.
Salinity levels (S)												
S1 (control)	83.98a	85.78a	84.88a	11.80a	12.93a	12.37a	29.13a	30.85a	29.99a	67.38a	69.98a	68.68a
S2	55.95b	56.72b	56.33b	6.52b	7.93b	7.23b	15.48b	16.30b	15.89b	39.20b	41.43b	40.32b
S3	45.03c	48.97c	47.00c	4.88c	6.42c	5.65c	11.40c	12.15c	11.78c	31.37c	30.90c	31.13c
F. test	*	**	**	*	**	**	*	*	**	**	*	*
Cultivars (C)												
Giza 125	65.48a	68.74a	67.11a	8.58a	9.72a	9.15a	20.70a	22.23a	21.47a	51.13a	52.43a	51.78a
Giza 132	57.83b	58.90b	58.37b	6.89b	8.47b	7.68b	16.64b	17.30b	16.97b	40.83b	42.44b	41.64b
F. test	**	*	*	**	**	**	*	*	*	*	*	*
Interaction (S*C)	NS	NS	NS	**	*	**	*	*	*	*	**	**

S1 (control): 1.2 dS m^{-1}; S2: 13.2 dS m^{-1}, S3: 20.5 dS m^{-1}.

Table 4. Effect of salinity levels and cultivars on leaf osmotic potential, leaf proline content and leaf Na$^+$ and K$^+$ content of barley plants at 105 days from sowing in the two seasons and their combined.

Factors	Leaf osmotic potential (-MPa)			Proline content mg g^{-1}FW			Na$^+$ content in leaves (mg g^{-1} DW)			K$^+$ content in leaves (mg g^{-1} DW)		
	2011/12	2012/13	Comb.	2011/12	2012/13	Comb.	2011/12	2012/13	Comb.	2011/12	2012/13	Comb.
Salinity levels (S)												
S1 (control)	0.540a	0.454a	0.497a	10.53c	8.37c	9.45c	7.47c	10.48c	8.97c	71.40a	66.22a	68.81a
S2	1.546b	1.346b	1.446b	24.53b	23.20b	23.87b	32.12b	36.13b	34.13b	58.30b	53.10b	55.70b
S3	2.004c	1.764c	1.884c	27.43a	25.03a	26.23a	47.60a	50.32a	48.96a	51.47c	44.00c	47.73c
F. test	*	**	**	**	**	**	**	*	**	*	*	*
Cultivars (C)												
Giza 125	0.982a	0.864a	0.923a	24.31a	22.23a	23.27a	25.97b	28.73b	27.35b	62.00a	55.72a	58.86a
Giza 132	1.744b	1.512b	1.628b	17.36b	15.50b	16.43b	32.16a	35.89a	34.02a	58.78b	53.16b	55.97b
F. test	**	*	*	**	*	**	*	**	**	*	*	*
Interaction (S*C)	*	**	**	*	**	**	*	*	*	**	*	**

S1 (control): 1.2 dS m^{-1}; S2: 13.2 dS m^{-1}, S3: 20.5 dS m^{-1}.

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Table 5. Effect of salinity levels and cultivars on yield measurements of barley plants at harvest in the two seasons and their combined

Factors	Number of spikes m^{-2}			1000-grain weight (g)			Grain yield (ardab fad^{-1})			Straw yield (t fad^{-1})		
	2011/12	2012/13	Comb.	2011/12	2012/13	Comb.	2011/12	2012/13	Comb.	2011/12	2012/13	Comb.
Salinity levels (S)												
S1 (control)	213.33a	218.67a	216.00a	40.68a	38.45a	39.57a	10.17a	11.00a	10.58a	2.56a	2.79a	2.67a
S2	124.67b	135.17b	129.92b	27.10b	25.05b	26.08b	6.32b	6.18b	6.25b	1.77b	2.00b	1.89b
S3	74.67c	86.67c	80.67c	18.17c	14.87c	16.52c	4.18c	3.85c	4.02c	0.92c	1.20c	1.06c
F. test	*	**	**	**	**	**	**	*	**	*	*	*
Cultivars (C)												
Giza 125	141.56a	149.89a	145.72a	29.79a	27.38a	28.58a	7.13a	7.34a	7.24a	1.81a	2.07a	1.94a
Giza 132	133.56b	143.78b	138.67b	27.51b	24.87b	26.19b	6.64b	6.68b	6.66b	1.68b	1.92b	1.80b
F. test	**	*	*	**	*	**	*	**	**	*	*	*
Interaction (S*C)	*	**	**	*	**	**	*	*	*	ns	ns	ns

S1 (control): 1.2 dS m^{-1}; S2: 13.2 dS m^{-1}, S3: 20.5 dS m^{-1}.

Table 6. Effect of salinity levels and cultivars on Na$^+$ and K$^+$ contents in grain of barley plants at harvest in the two seasons and their combined

Factors	Na$^+$ (µg g^{-1}DW)			K$^+$ (µg g^{-1}DW)		
	2011/12	2012/13	Comb.	2011/12	2012/13	Comb.
Salinity levels (S)						
S1(control)	48.3c	61.3c	54.8c	1010.0a	865.0a	937.5a
S2	78.3b	88.0b	83.2b	805.0b	651.7b	728.3b
S3	116.7a	138.0a	127.3a	595.0c	443.3c	519.2c
F. test	*	*	*	*	**	**
Cultivars (C)						
Giza 125	68.9b	83.6b	76.2b	832.2a	686.7a	759.4a
Giza 132	93.3a	108.0a	100.7a	774.4b	620.0b	697.2b
F. test	*	*	*	**	*	*
Interaction (S*C)	**	*	**	*	*	*

S1 (control): 1.2 dS m^{-1}; S2: 13.2 dS m^{-1}, S3: 20.5 dS m^{-1}.

of salinity (S3), indicating that this cultivar had experienced lower injury and showed higher tolerance to salt stress. Because of the fundamental relationship between photosynthesis and yield, there is considerable interest to determine the association between photosynthetic pigments (which influenced directly on photosynthesis) and grain yield. Total chlorophyll (a+b) correlated significantly with grain yield for barley under salinity and normal conditions. This indicates when stress is involved; ability of the plants to continue a relatively high rate of photosynthesis activity may very well contribute to yield.

Relative Water Content (RWC %)

Relative water content was determined to give an indication on the plant water dehydration status during exposure to salinity. Under normal salinity condition (S1), the two cultivars were maintained a maximum relative water content (RWC) (69.18 and 68.18% in Giza 125 and 132, respectively) without significant differences between them. Exposure to salinity reduced the RWC by about (29 to 46% in Giza 125) and (53 to 64% in Giza 132) under S2 and S3 levels, respectively compared to normal level of salinity as shown in (Table 7). Moreover, it can be concluded that Giza 125 cv plants which tended to keep up their RWC under salinity stress, they acquire their tolerance from great solute accumulation and metabolites, hence, osmotic adjustment kept higher the RWC.

Leaf osmotic potential (-Ψs Mpa)

Leaf osmotic potential (-Ψs) is the main component of physiological machinery, by which plants respond to soil salinity stress. Leaf osmotic potential taken the negative sign of the figures into account, were higher under stress conditions than non-stress conditions. It was the most physiological trait advocated for contribution to salinity tolerance between cultivars. As shown in Table 7, leaves of the two cultivars showed normal osmotic potential (-0.536 and -0.466 Mpa in cv. Giza 125 and 132, respectively) under low level of salinity (S1) without significant differences between them. Increment of salt levels led to diminish the osmotic potential to higher negative values (0.8 to 1.4 time in Giza 125 cv.) and (3.1 to 4.3 times in Giza 132 cv.) when exposed to high levels (S2 and S3) of salinity, respectively compared to

low levels of salinity (1.2 ds/m). Leaf osmotic potential has been proposed as an important response to salt deficit. On the other hand, it has been recognized for a long time as an important mechanism in salinity tolerance. Increases in the concentration of solutes in solution lead to an increase in osmotic potential

Proline Content

Maximum contents (31.25 and 28.9 mg g⁻¹FW) of proline were recorded in leaves of Giza 125 cv. under salinity levels of 20.5 and 13.2 ds/m, respectively with significant differences between them. The content of proline was increased by about 2 to 2.2 times in leaves of Giza 125 cv. and only by about 1 to 1.3 time in Giza 132 cv. under the S2 and S3 levels of salinity, respectively (Table 7). It is clear that Giza 125 cv. possesses different mechanisms to be more tolerant to salinity than Giza 132 cv., indicating that accumulated proline acts as a compatible solute regulating and reducing water loss from the cell during episodes of water deficit for the salt treatment.

Leaves Na⁺ and K⁺ ions contents

Insignificant differences in Na⁺ content in leaves of the two cultivars (8.2-9.8 mg g⁻¹ DW in both Giza 125 and 132 cvs., respectively) under low level of salinity (S1) were observed. Exposure to high level of salts increased the accumulation of Na⁺ content by about 2.8 to 3.5 times and 2.8 to 4.6 times under S2 and S3 levels in both Giza 125 and 132 cvs., respectively. However, normal K⁺ homeostasis content (68.5 – 69.1 mg g⁻¹ DW in Giza 125 and 132, respectively) was also observed in both two cultivars under minimum level of salinity. Moreover, this normal homeostasis content of K⁺ was decreased by about (15 – 28%) in Giza 125 and by (23 – 34%) in Giza 132 under high level of salinity conditions S2 and S3, respectively (Table 7). It can be concluded that Giza 125 was accumulated more content of K⁺ and excluded Na⁺ compared to Giza 132 under high salinity conditions. Salt tolerance is generally considered to be associated with Na⁺ ion exclusion during growth under saline condition. Na⁺ toxicity is strongly linked to plant's ability to maintain uptake and within plant distribution of K⁺. Also, grain yield was correlated with Na⁺ exclusion and associated enhanced K⁺/Na⁺ discrimination.

of salinity (S3), indicating that this cultivar had experienced lower injury and showed higher tolerance to salt stress. Because of the fundamental relationship between photosynthesis and yield, there is considerable interest to determine the association between photosynthetic pigments (which influenced directly on photosynthesis) and grain yield. Total chlorophyll (a+b) correlated significantly with grain yield for barley under salinity and normal conditions. This indicates when stress is involved; ability of the plants to continue a relatively high rate of photosynthesis activity may very well contribute to yield.

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low levels of salinity (1.2 ds/m). Leaf osmotic potential has been proposed as an important response to salt deficit. On the other hand, it has been recognized for a long time as an important mechanism in salinity tolerance. Increases in the concentration of solutes in solution lead to an increase in osmotic potential

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Maximum contents (31.25 and 28.9 mg g^{-1} FW) of proline were recorded in leaves of Giza 125 cv. under salinity levels of 20.5 and 13.2 ds/m, respectively with significant differences between them. The content of proline was increased by about 2 to 2.2 times in leaves of Giza 125 cv. and only by about 1 to 1.3 time in Giza 132 cv. under the S2 and S3 levels of salinity, respectively (Table 7). It is clear that Giza 125 cv. possesses different mechanisms to be more tolerant to salinity than Giza 132 cv., indicating that accumulated proline acts as a compatible solute regulating and reducing water loss from the cell during episodes of water deficit for the salt treatment.

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Table 7. The interaction effect between salinity levels and cultivars on some growth analysis, proline content and Na $^+$ and K $^+$ content in leaves of barley plant at 105 days from sowing (combined data)

Salinity levels (S)	Cultivars (C)	Flag leaf blade area (cm^2)	Total chlorophyll (mg 100g^{-1} FW)	Relative water content (%)	Leaf osmotic potential (-Mpa)	Proline content mg g^{-1}FW	Na$^+$ (mg g-^{1} DW)	K$^+$(mg g-^{1} DW)
S1 (control)	Giza 125	11.87a	29.62a	69.18a	0.536a	9.62e	8.18e	68.53a
	Giza 132	12.87a	30.37a	68.18a	0.466a	9.28e	9.77e	69.08a
S2	Giza 125	8.67b	19.80b	48.82b	0.964b	28.95b	30.98d	58.40b
	Giza 132	5.78d	11.98d	31.82d	1.930d	18.78d	37.27c	53.00c
S3	Giza 125	6.92c	14.98c	37.35c	1.280c	31.25a	42.88b	49.65d
	Giza 132	4.38e	8.57e	24.92e	2.486e	21.22c	55.03a	45.82e

S1 (control): 1.2 dS m^{-1}; S2: 13.2 dS m^{-1}, S3: 20.5 dS m^{-1}.

Number of Spikes m^{-2}

The two barley cultivars showed differential response to the increase of salinity level. In this connection, Giza 132 cv. significantly produced the highest number of spikes m^{-2} (about 219) compared with Giza 125 cv. (about 213) under the low level of salinity. On the other hand, under the moderate and the high salinity levels, the number of spikes m^{-2} was significantly increased with Giza 125 cv. compared with Giza 132 cv. Under the 13.2 and 20.5 ds/m salinity levels, gradual reduction of number of spikes m$^-$^{2} by 37- 58 % in Giza 125 cv. and 43-67% in Giza 132 cv. was observed, in respective order (Table 8).

1000- Grain Weight

The two cultivars under study recorded significantly the heaviest weight of 1000- grains (38.8 and 40.3 in Giza 125 and 132, respectively) under normal level of salt levels without significant differences between them (Table 8). Reduction of weight of 1000-grains was more pronounced in Giza 132 cv. (41 and 65%) compared with Giza 125 cv. (27 and 52%) under S2 and S3 levels, respectively.

Grain Na$^+$ and K$^+$ Ions Contents

Table 8 indicated that very low content of Na$^+$ (about 50- 60 μg g^{-1}DW) was recorded in grains of the two cultivars (Giza 125 and Giza 132, respectively) grown under normal conditions of salinity without significant differences between them. Also, this amount was increased by about (0.5 – 1 time in Giza 125 cv.) and (0.5 and 1.5 time in Giza 132 cv.) under high salinity levels S2 and S3, respectively. On contrary, grains of the two cultivars maintained high significant amount of K$^+$ which amounted to 918.3 – 956.7 μg g^{-1}DW, in Giza 125 and 132, respectively, under normal conditions. Meanwhile, this amount was decreased again by about 13 -39% in Giza 125 and 31- 51 % in Giza 132, under S2 and S3 of salinity levels, respectively. It could be concluded that grains of Giza 125 cv. accumulated more K$^+$ but less Na$^+$ ions compared to grains of Giza 132 cv. under high salinity level.

Grain Yield

Under normal level of salinity, the highest grain yield as cited in Table 8 was obtained from Giza 132 cv. (10.9 ardab fad^{-1}) compared with Giza 125 cv. (10.27 ardab fad^{-1}) with significant differences between them but with different magnitudes. Increasing salinity levels gradually and significantly reduced grain yield in both cultivars but with different magnitudes. Reduction of grain yield of Giza 125 cv. was by about (0.3 – 0.6 time) but in Giza 132 cv. by about (0.5 – 0.7 time) under S2 and S3 level of salinity, respectively. Under high salinity levels (S2 - S3), grain yield was significantly ameliorated by Giza 125 (6.92 – 4.53 ardab fad^{-1}) in comparison with Giza 132 (5.58 – 3.5 ardab fad^{-1}).

Table 8. The interaction effect between salinity levels and cultivars on yield and its components of barley at harvest (combined data)

Salinity levels (S)	Cultivars (C)	Number of spikes m^{-2}	1000-grain weight (g)	Grain yield (ardab fad^{-1})	Na$^+$ content in grain (µg g^{-1}DW)	K$^+$ content in grain (µg g^{-1}DW)
S1 (control)	Giza 125	213.17b	38.80a	10.27b	49.0e	918.3a
	Giza 132	218.83a	40.33a	10.90a	60.7e	956.7a
S2	Giza 125	135.17c	28.17b	6.92c	75.7d	795.0b
	Giza 132	124.67d	23.98c	5.58d	90.7c	661.7c
S3	Giza 125	88.83e	18.78d	4.53e	104.0b	565.0d
	Giza 132	72.50f	14.25e	3.50f	150.7a	473.3e

S1 (control): 1.2 dS m^{-1}; S2: 13.2 dS m^{-1}, S3: 20.5 dS m^{-1}.

Protein Profile

Fig. 1 showed protein profile of two cvs. of barley leaves under salinity conditions. Densitometer analysis (Fig. 2) of water soluble protein fractions in SDS-PAGE recorded that molecular weight of protein bands ranged from 152 to 4 kDa as shown by protein marker. Number of protein bands under control conditions reached to 22 and 25 in both Giza 125 and 132, respectively. Under high levels of salinity S2 and S3, this number of bands was reduced to 20 and 20 bands in Giza 132 in compared to 29 and 23 bands in Giza 125 under the same conditions, respectively. Under S1, S2 and S3 levels, higher optical density of bands which have a high molecular weight 82-87 kDa were detected in both cultivars for bands number 7, 6, 6 in Giza 132 and bands number 6, 6, 5 in Giza 125, respectively. Also, higher optical density of low molecular weight bands (25 kDa) was detected in both cultivars under all salinity levels for bands number 19, 14, 12 in Giza 132 and bands number 16, 23, 16 in Giza 125 under S1, S2 and S3 levels, respectively. Under high level of salinity S3, disappear of bands with molecular weight 67 and 34 kDa in Giza 132 and founded in Giza 125 can use as molecular marker for salt tolerance in barley. In the meantime, similarity % (Fig. 3) among both cultivars of barley varied according to salt levels. The highest value of similarity (85.71 and 80.93%) was recorded between both cultivars under high level of salinity 20.5 and low level 1.2ds/m, respectively, but the lowest ones (77.92%) was found under moderate level of salinity (13.2 ds/m).

Iso-Enzyme Profile

Esterase

Table 9 and Fig. 4 showed that, the two cultivars under study differed in 11 isoforms of esterase isoenzymes under the three levels of salinity. In total, under low salinity level, 9 isoenzymes of esterase were detected in leaves of Giza 132 cv. compared with only 7 bands in Giza 125 cv. Under moderate salinity level, 8 isoforms of esterase were expressed in Giza 132 compared with 7 bands in Giza 125. Meanwhile, 11 esterase isoenzymes were resolved in barley Giza 132 in compared with 6 isoesterase forms in Giza 125 under high level of salinity. Four isoesterase (EST 1, 2, 6 and 7) were resolved in Giza 132 cv. under high level of salinity and disappeared in Giza 125 cv. under all salinity levels.

Peroxidase (POD)

Table 9 and Fig. 5 revealed that the two cultivars of barley under investigation differed in 6 isoformes of peroxidase under all three levels of salinity. Plants of Giza 125 cv. were expressed 6 isoperoxidases bands under three levels of salinity S1, S2 and S3 compared with 5 isoperoxidases bands were detected in leaves of Giza 132 cv. under all pervious circumstances. POD6 as unique isoform was resolved in Giza 125 and not expressed in Giza 132 under all salinity levels.

Table 9. Ideogram of esterase (EST) and peroxidase (POD) isoenzymes of two cultivars of barley Giza 125 and 132 under different salinity levels.

	Esterase (EST)							Peroxidase (POD)					
	Giza 125			Giza 132				Giza 125			Giza 132		
	Salt levels							Salt levels					
Isobands	S1	S2	S3	S1	S2	S3	Isobands	S1	S2	S3	S1	S2	S3
EST 1	-	-	-	-	-	+	POD 1	+	+	+	+	+	+
EST 2	-	-	-	-	-	+	POD 2	+	+	+	+	+	+
EST 3	+	+	+	+	+	+	POD 3	+	+	+	+	+	+
EST 4	+	+	+	+	+	+	POD 4	+	+	+	+	+	+
EST 5	+	+	-	+	+	+	POD 5	+	+	+	+	+	+
EST 6	-	-	-	+	-	+	POD 6	+	+	+	-	-	-
EST 7	-	-	-	+	+	+							
EST 8	+	+	+	+	+	+							
EST 9	+	+	+	+	+	+							
EST 10	+	+	+	+	+	+							
EST 11	+	+	+	+	+	+							
Total	7	7	6	9	8	11		6	6	6	5	5	5

S1 (control): 1.2 dS m^{-1}; S2: 13.2 dS m^{-1}, S3: 20.5 dS m^{-1}.
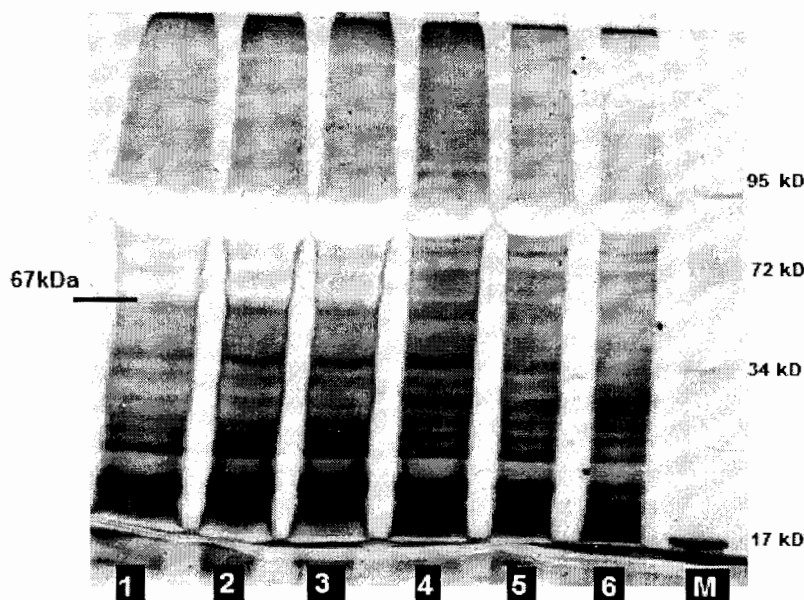
Fig. 1. SDS- PAGE of water soluble proteins extracted from leaf of barley cultivars Giza 125 and 132 , M marker protein, Lane 1, 2, 3 for Giza 125, Lane 4, 5, 6 for Giza 132 under 1.2, 13.2 and 20.5 ds/m, respectively
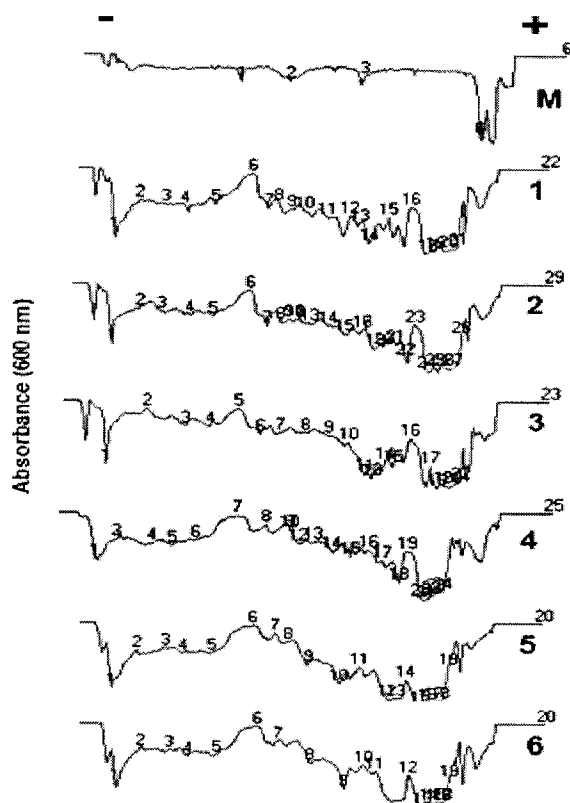
Fig. 2. Densitometric tracing of water soluble proteins bands isolated from leaf of barley cultivars Giza 125 and 132 , M marker protein, 1, 2, 3 for Giza 125, 4, 5, 6 for Giza 132 under 1.2 , 13.2 and 20.5 ds/m, respectively
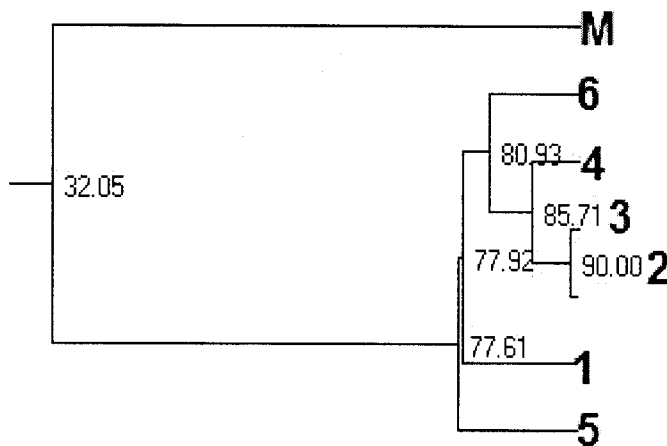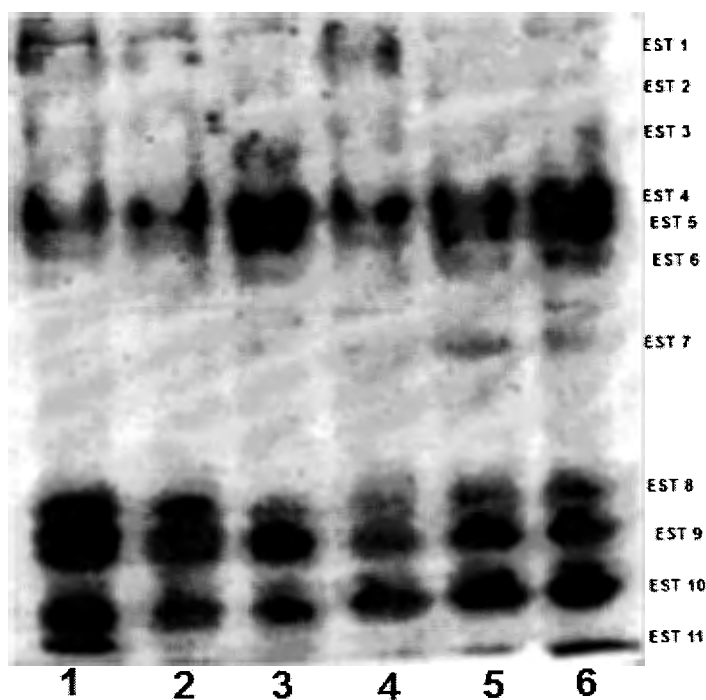

Fig. 3. Cluster analysis on bases of SDS-PAGE between two barley cultivars Giza 125 and 132 , M marker protein, 1, 2, 3 for Giza 125, 4, 5, 6 for Giza 132 under three levels of salinity 1.2 , 13.2 and 20.5 ds/m, respectively

Fig. 4. PAGE of esterase (EST) isoenzymes of two barley cultivars, 1, 2, 3 for Giza 125, 4, 5, 6 for Giza 132 under three levels of salinity 1.2, 13.2 and 20.5 ds/m, respectively
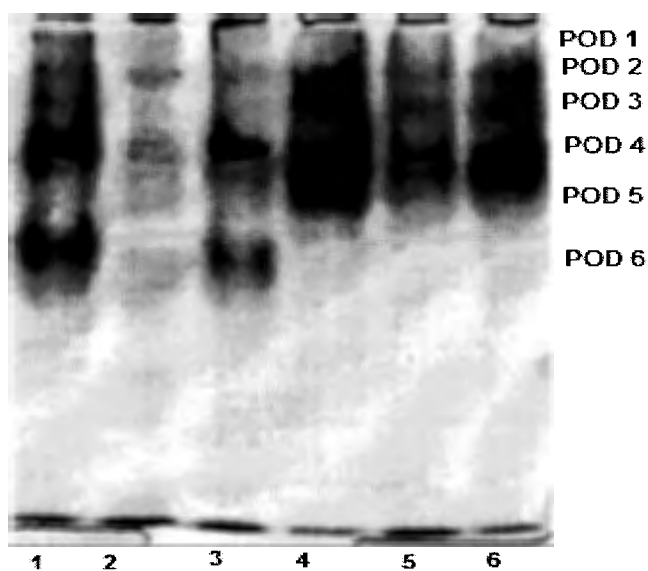
Fig. 5. PAGE of peroxidase (POD) isoenzymes of two barley cultivars, 1, 2, 3 for Giza 125, 4, 5, 6 for Giza 132 under three levels of salinity 1.2, 13.2 and 20.5 ds/m, respectively

DISCUSSION

Comparison experiment between two cultivars of barley was conducted to estimate their productivity and their tolerance to salinity. Giza 125 and 132 were cultivated in new salty soil containing different concentrations of salt ions, Na^+, Cl^-, SO_4^{-2}, Mg^{2+}, Ca_2^+, K^+, CO_3^- and HCO_3^- with electrical conductivity of 1.2, 13.2 and 20.5 ds/m (as shown in Table 1a) and irrigated with mixed water (Nile + sewage, 1:1) from Al-Salam Canal with EC: 1.9 ds m^{-1} (Table 1b). The electrical conductivity (EC) in ds/m values were altered by 0.30 -0.32 in L1, 0.60 - 0.50 in L2 and 0.70- 0.60 in L3 during 2011/12 and 2012/13 seasons, respectively (Table 2). Also, the EC of irrigation water was changed between 0.15- 0.10 during 2011/12 and 2012/13 seasons, respectively (Table 2).

The present results showed that the two cultivars under study revealed different responses to salt stress, where Giza 125 cv. was more tolerant to salt stress than Giza 132 cv. in most growth, production and biochemical parameters. The gradual decrease of the plant height (Table, 7) may be due to accumulation of inhibitors phytohormone, abscisic acid, and decline of both promoting phytohormones, cytokinins and indol acetic acid (Shakirova et al., 2003) or reduction of cytokinin export from root to the shoot as occurred in different cereals plants under high salinity levels (Kuiper et al., 1990).

By increasing of soil salinity, reduction of both leaf area and relative water content (Table, 7) in cv. Giza 132 compared to cv. Giza 125 is primarily due to the osmotic effect of the salt around the roots (Tables 1 and 2). Osmotic potential of the three experimental locations was about -0.0432 , -0.4752 and -0.738 Mpa for the three salinity levels, respectively. Under normal condition of salinity (1.2 ds m^{-1}), the osmotic potential (Ψ_s) in flag leaf of Giza 132 and Giza 125 cvs. was more negatively by about 10 and 12 than the osmotic potential (Ψ_s) of soil solution. This may explain the high potential of barley to salt tolerance compared to other crops. The osmotic potential (Ψ_s) of flag leaves was negatively increased by only 3.1, 1.7 times in Giza 125 and 6.2, 3.2 times in Giza 132 under moderate and high salinity levels, respectively.

Also, previous investigations obvioused that, salt tolerance of barley under field conditions may derive partly from its rapid growth and fast phenological development, leading to an early maturity date (Maas and Hoffman, 1977). Under salt stress, osmotic potential of plants becomes more negatively increased, whereas turgor pressure increases (Morales et al., 1998). Under saline conditions, the present results confirmed that leaves of the more tolerant Giza 125 cv. had lower osmotic potential values than the sensitive Giza 132 cv. (Table, 7). The tolerant cultivar plants adjusted their osmotic potential to a moderate value (-1.280 Mpa) compared to the sensitive ones (-2.486 Mpa) under high levels of salinity. Lowering of osmotic potential values in Giza 132 cv. contributed with more accumulation of Na^+ ions and less accumulation of K^+ and proline (Table, 7). On the contrary, Giza 125 may be having a higher osmotic potential contributed with more accumulation of osmolytes as K^+ and proline which induced improvement in plant water status. On the other hand, high Na^+ and Cl^- uptake competes with the uptake of other nutrient ions, leading to K^+ deficiency. Increased treatment of NaCl induces a specific ion effect which leads to an increase in Na^+ and Cl^- and a decrease in Ca^{2+}, K^+, and Mg^{2+} levels in a number of plants (Khan et al., 1999). Also, Bavei et al. (2011) found that, clear decline of K^+ and Ca^{2+} concentrations and increase of Na^+ and proline contents were observed in the root and leaf tissues at each NaCl concentration in sensitive sorghum varieties during the NaCl treatment. The genes associated with a locus Knal appears to be absent in barley, as judged by the high Na+ and low K+ concentrations compared with wheat (Gorham et al., 1990).

Aloni and Rosenshtein (1984) reported that proline plays an important role as osmoregulator under drought and salinity conditions, proteins stabilizer, prevention of heat denaturation of enzymes and conservation of nitrogen and energy for a post-stress period. The present results demonstrated that the tolerant Giza 125 cv. may be tended to use relatively low energy consumed-compatible solute as K^+ ions (58.3-51.47 mg DW^{-1}) than high energy-organic solutes such as proline (24.53–27.43 mg $g^{-1}FW$).

Low concentration of Na^+ (104 µg g^{-1} DW) was observed in barley grains under high levels of salinity, which represented benefit for human health, especially for high pressure patients. Na^+ ions were ascending in xylem with transpiration stream, then accumulated in leaves and in contrary, not transported across the phloem and not accumulated in the reproductive parts (Taiz and Zeiger, 2002).

Reduction of cell elongation and also cell division lead to slower leaf growth and hence smaller final size (Berkowitz, 1998). Decreasing of vegetative parameters due to increase salinity may be a result of a combination of osmotic and specific ion effects of Cl^-, K^+ and Na^+ on enzymes activity (Munns, 2002).

Salinity decreases leaf photosynthetic rate due to several factors as dehydration of cell membranes which reduce their permeability to CO_2, salt toxicity, reduction of CO_2 uptake because of hydroactive closure of stomata, enhanced senescence induced by salinity, changes of enzyme activity induced by changes in cytoplasmic structure, and negative feedback by reduced sink activity (Iyengar and Reddy, 1996). Decreasing of photosynthetic rate, LA and leaf water relation was contributed with reduction of spike number/m^2, 1000 grain weight and grain yield per fad., in the sensitive Giza 132 compared to the other salt-tolerant cultivar (Table 8). At S3 level (20.5 ds m^{-1}), yield was reduced by about 56% in Giza 125 cv. and 68% in Giza 132 cv.

Salt stress causes oxidative stress because of the formation of reactive oxygen species (ROS) such as superoxides and hydroxy and peroxy radicals. The excess of ROS causes, chlorophyll degradation, membrane disfunction and cell death (Bohnert and Jensen, 1996). Therefore, the total chlorophyll content of leaves in the more tolerant Giza 125 was more stable than in the less tolerant one Giza 132 (Table, 7). Actually, reduction in chlorophyll content accompanied with decreases of Ca^{2+} and Mg^{2+} contents of leaves which excluded out the cell due to increase of Na^+ concentration (Parida *et al.*, 2004). Also, the decrease in chlorophyll may be due to the formation of proteolytic enzymes such as chlorophyllase, which is responsible for chlorophyll degradation.

The response of plants to salinity is based on the action of many defense proteins/enzymes. Salt concentrations higher than 400 mM NaCl inhibit most enyzmes because of the perturbation of the hydrophobic-electrostatic balance between the forces maintaining protein structure (Serrano *et al.*, 1999).

Detection of about 23 or 29 protein bands in the tolerant Giza 125 cv. compared to only 20 of protein bands in Giza 132 cv., meaning that the more tolerant cultivar has highly protective mechanism to their protein (Figs. 1 and 2), and this protection is through synthesis new protective protein with low molecular weight as late embryogenesis abundant proteins, osmotein, germine and dehydrins. Salinity induces six new proteins in roots of barley, which are of low molecular weight, 24 to 27 kDa, with an isoelectric point of 6.1 to 7.6. In contrast to roots, five new shoot proteins are induced whose molecular weights and isoelectric points fall within the range of 20–24 kDa and 6.3–7.2, respectively. In contrary, salinity inhibits the synthesis of a majority of shoot proteins (Ramagopal, 1987). Under high level of salinity S3, bands with molecular weight of 67and 34 kDa was disappeared in the sensitive Giza 132 and founded in the tolerant Giza 125 which can be used a molecular marker for salt tolerance of new salt-tolerant barley (Figs. 1 and 2).

Presented results demonstrated that esterase isoenzymic pattern could severe as useful molecular indicators of salinity sensitivity. Activity of esterase as a hydrolysis enzyme especially in lipids of cell membrane under stress was increased. More expression of isoesterases in the sensitive Giza 132 cv., reached to 11 isoforms, compared to only 7 isoenzymes in the more tolerant Giza 125 cv., may be change the plasma membrane integrity (Fig. 4). About 4 new isoesterases in sensitive was found and can use as selective marker between new salt-tolerant strains of barley. Esterase activities in shoots decreased compared to root in response to increased saline treatments. In total, 12 and 14 esterase isoenzymes were resolved in halophytes *Centaurea ragusina* leaves and roots, respectively. Results demonstrate that esterase activities and their isoenzymic patterns could

serve as useful bioindicators of salinity (Radi and Pevalek-Kozlina, 2010). NaCl-induced stress stimulate 4–5 isoenzymes of esterase in peanut roots versus three new isoenzymes in its leaves after two-week period (Hassanein, 1999).

In contrary, the tolerant Giza 125 cv. was expressed new isoperoxidases isoform (POD6) compared to the sensitive Giza 132 cv. This new isoform can increase the antioxidant potential capacity of plants to scavenge the excess of H_2O_2 produced under salinity stress. Genetic modified crops with high levels of antioxidants capacity or overexpression of compatible solutes showed high tolerance to salinity (Hayashi and Murata, 1998). These new unique band can use a useful molecular marker for salt tolerance. Goudarzi and Pakniyat (2009) found that proline, protein contents and peroxidase activity were increased in most tolerant cultivars of wheat grown under 6.8 and 13.8 ds/m NaCl.

Previous results showed that esterases isoforms are more suitable biomarkers of salt stress than isoperoxidases because of its high numbers of specific 11 bands detected in barley than 6 of isoperoxidases bands.

Conclusion

Although barley is a tolerant crop to salinity, but cultivars vary in their tolerance capacity. The increase of salinity level decreased plant height, flag leaf blade area, the number of spikes m^{-2}, weight of 1000 grains and grain yield. Total chlorophylls, relative water content, leaf osmotic potential, proline and K+ contents as biochemical parameters contributed with salt tolerance. Protein profile, isoenzymes of esterase and peroxidase were fine molecular markers to select the new salt-tolerant genotypes of barley.

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تقييم المحصول وتحمل الملوحة لصنفين من الشعير فى ثلاث مناطق مختلفة فى مستوى الملوحة

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أجريت هذه الدراسة خلال موسمى ٢٠١١/١٢ و٢٠١٢/١٣ للمقارنة بين صنفين من الشعير المنزرع في ثلاث مواقع تختلف الأراضى فيها فى مستوى الملوحة من أجل تقييم إنتاجها ودرجة تحملها للملوحة، ولذلك أجريت تجربة حقلية باستخدام تصميم القطاعات كاملة العشوائية فى ثلاث مواقع في القنطرة شرق ومنطقة سهل الطينة بمحافظة الإسماعيلية وبور سعيد اختلفت في مستويات الملوحة (١,٢ ــ ١٣,٢ ــ ٢٠,٥ ديسيسيمينز/م) وصنفين من الشعير جيزة ١٢٥ و١٣٢ حيث حللت كل تجربة منفردة ثم تم إجراء التحليل التجميعى للمواقع لايجاد تاثير الملوحة و التفاعل بين الأصناف والملوحة و ذلك بعد إجراء اختبار التجانس لبارتلت، تم تقييم بعض المقاييس المورفولوجية والفسيولوجية والإنتاجية عند ١٠٥ يوم من الزراعة وعند الحصاد، تقييم بعض الصفات "مثل ارتفاع النبات ومساحة نصل ورقة العلم ومحتوى الكلوروفيل الكلى والماء النسبى والجهد الازموزى وكمية البرولين وكمية البوتاسيوم والصوديوم وعدد السنابل/م٢ ووزن ١٠٠٠ حبة ومحصول الحبوب" أشار إلى أن الصنف جيزة ١٢٥ كان أعلى تحملا للملوحة من الصنف جيزة ١٣٢، بوجه عام ارتبط تحمل الصنف للملوحة بالمستوى العالي لمحتوى الكلوروفيل الكلى والماء النسبى والجهد الازموزى وكمية البرولين وكمية البوتاسيوم بالأوراق، رغم تراكم الصوديوم فى الأوراق عند ١٠٥ يوم من الزراعة (٥٥ ملجرام/جم مادة جافة) إلا أن محتوى الحبوب منه كان اقل عند الحصاد (١٥٠ ميكرو جرام/ جم مادة جافة)، وقد أمكن تحديد حوالى ٢٣ـ٢٩ حزمة بروتينية من البروتين الذائب فى الماء فى الصنف المقاوم جيزة ١٢٥ مقارنة بـ ٢٠ حزمة فقط فى الصنف جيزة ١٣٢ عند المستويات العالية من الملوحة، وجود الحزمتين البروتينيتين ذات الوزن الجزيئى ٦٧ و ٣٤ كيلودالتون فى الصنف المقاوم جيزة ١٢٥ يمكن استخدامهما كحزمتين مميزتين للصنف المقاوم للملوحة فى الشعير، عبر الصنف الحساس جيزة ١٣٢ بـ ٩ـ١١ حزمة من مشابهات إنزيم الاستيريز مقارنة بـ ٧ حزم فقط فى الصنف المقاوم للملوحة جيزة ١٢٥، ومن ناحية أخرى، تميز الصنف المقاوم للملوحة جيزة ١٢٥ بوجود ٦ حزم من مشابهات إنزيم البيروكسيديز مقارنة بـ ٥ حزم فقط فى الصنف الحساس للملوحة جيزة ١٣٢، يمكن التوصية بإمكانية الاعتماد على الحزم المميزة من البروتين ومشابهات الإنزيمات خاصة الاستيريز (لزيادة عدد الحزم) فى انتخاب السلالات المتحملة للملوحة والمرتبطة بالإنتاجية العالية فى الشعير والتى تتميز بعدد أكبر من السنابل/م٢ وارتفاع عدد الحبوب ووزن ١٠٠٠ حبة وفى النهاية زيادة محصول القش والحبوب للفدان.