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EMINO AMINO ACIDS ACCUMULATION AND ITS IMPLICATION IN BARLEY TOLERANCE TO SALT STRESS UNDER RAS SUDR CONDITIONS, SOUTH SAINI, EGYPT

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ABSTRACT

This study was conducted to evaluate biochemical changes induced by foliar application with proline (control, 20, 40 and 60 ppm) on barley plants (Giza123 and Giza126) under saline conditions (4557 and 8934 ppm) at Ras Sudr. Proline had a positive effect on growth, yield and biochemical components in barley cultivars. Giza123 was better than Giza126. With respect to free amino acids, data showed that thirty three acids were detected in two barley cultivars. The most abundant amino acids noticed were asparagine, proline, alanine, cystine, γ-aminobutyric and lysine. Also, proline treatments led to decrease of malondialdehyde content and quaternary ammonium compounds (glycinebetaine and choline) in barley plants compared with the control. On contrary, the accumulation of quaternary ammonium compounds took the reverse effect with salinity. Proline treatments had a positive effect on antioxidant enzymes under two salinity levels. In this regard, superoxide dismutase and catalase patterns revealed the presence of about five bands for the two barley cultivars under low and high salinity as well as it was increased in band intensity at all samples under high salinity compared with low salinity. Electrophoretic behavior of soluble proteins showed the presence of 12-23 bands with 18-229 kDa. Bands of molecular masses 20, 25, 37, 56, 72 and 149 kDa in two barley cultivars were absent in all proline treatments under low salinity level and accumulated with all doses of proline and control under high salinity level and thus can be used as biomarker to salt tolerance. We can benefit from current study in alleviate the adverse effects of saline stress on barley plants under Ras Sudr conditions, by activating the role of induced resistance using proline which had a positive effect on most of the biochemical components and barley grain yield.

Key words: Salinity, barley, emine acids, free amino acids, antioxidant enzymes, malondialdehyde, quaternary ammonium compound.

INTRODUCTION

Salt stress imposes a major environmental threat to agriculture by limiting plant growth and reducing crop yield. The increased salinization of arable land is expected to have global effects, resulting in 30% land loss within the next 25 years (Wang *et al.,* 2003). Therefore, the efforts to increase salt tolerance of crop plants bear remarkable importance for sustainable agriculture. Salinity affects plant growth and development by imposing osmotic stress on plants, causing specific ion (Na^+) toxicity, affecting activity of major cytosolic enzymes by disturbing intracellular potassium homeostasis and causing oxidative stress in plant cells (Marschner, 1995; Sairam and Srivastava, 2002; Cuin and Shabala, 2007; Chen *et al.,* 2007). This stimulates the generation of active oxygen species, such as singlet oxygen, superoxide

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anion, hydrogen peroxide and hydroxyl radical. These oxygen species are highly cytotoxic and can seriously react with vital biomolecules such as lipids, proteins, nucleic acids, *etc.*, causing lipid peroxidation, protein denaturing and DNA mutation, (Miller *et al*., 2008; Bose *et al*., 2013; Maksimovic *et al*., 2013; Adem *et al*., 2014; Hendawey, 2015). In this concern, reactive oxygen species can be generated by the direct transfer of the excitation energy from chlorophyll to produce singlet oxygen. In addition, hydrogen peroxide is a powerful inhibitor of the Calvin cycle in chloroplasts. Plants protect themselves from the harmful effects of oxidative stress by produce some defense mechanisms. Reactive oxygen species (ROS) scavenging is one of the common defense responses against salinity stress. ROS scavenging depends on the detoxification mechanism provided by an antioxidants system (enzymatic and non-enzymatic), (Molassiotis *et al.*, 2006; Noreen and Ashraf, 2009; Joseph and Jini, 2010; Bahari *et al.*, 2013).

Barley (*Hordeum vulgare* L.) is one of the important cereal crops in Egypt and can be grown in the arid and semiarid regions which affected by salinity such as Sinai Peninsula, which represent 6% (60000 Km^2) of the total area of Egypt, for the purposes of forage and grain crop (Al-Karaki, 2001). The rainfall or the existing fresh water in this region is limited, so irrigation depends mostly on underground water as well as the soil showed to be saline and highly calcareous. In Egypt, barley is the main crop and widely grown in the rainfed areas of the north coastal region and in the newly reclaimed lands with saline soils (Ceccarelli, 2008). Most cereals including barley are reported to be more salt tolerant at germination than seedling stage. Therefore seedling stage is relatively the most sensitive growth stage determining the plant stand density, which affects the final yield. The modern trends to push the plants for salt tolerance (oxidative stress) is the use of some environmentally safe materials such as amino acids especially proline. There are many studies in this field which emphasizes the important role of proline to enhance plants against salt stress (Zaki and Radwan, 2011; Talat *et al*., 2013; Hendawey, 2015). The research aimed to study of proline accumulation and its implication in barley tolerance to salt stress.

MATERIALS AND METHODS

Field Experiments

Two field experiments were carried out during 2011/2012 and 2012/2013 seasons at Agricultural Experimental Station of Desert Research Center (DRC) Located in Ras-Sudr, South Sinai Governorate, Egypt. To study the effects of variable salinity of irrigation water with foliar application of proline on two barley genotypes. The grains of barley cultivars (Giza 126 and Giza 123) were obtained from the Agricultural Research Center, Giza. Barley grains were sown on second week of November in both seasons. Recommended fertilization for this type of soil and other agricultural practices were applied according to Desert Research Center as recommended for the ordinary barley fields in the experimental location. The chemical analysis of irrigation water and soil were presented in Table 1. Treatments were arranged in split split plot design with three replicates. The experiment included sixteen treatments; *i.e.* two salinity levels of irrigation water (4557 and 8934ppm). Four foliar application of proline (control, 20, 40 and 60 ppm) and two barley cultivars (Giza 126 and Giza 123). Each treatment was sprayed on barley plants at rate of 400 liter/faddan after 30 and 45 days from sowing. Tween 20 was used as wetting agent at 0.05%.Three plant samples were taken randomly from each treatment during the experiment of each season. Two samples of fresh plants were collected after 45 and 60 days after sowing to determine some growth parameters (plant height, fresh and dry weights) and some biochemical constituents (protein patterns, isozymes, free amino acids and malondialdehyde). Then, dried till constant weight representing dry weight. Dry samples were ground to fine powder and tested for quaternary ammonium compounds (glycinebetaine and choline). The third one, represented by grain, was taken after harvesting (145 days from sowing) to determine grain, straw and biological yields.

Table 1. Water and soil chemical analysis

Level	pH	EC	Cations (meq/l)				Anions (meq/l)			
		ppm	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{-}	HCO_3^{-}	Cl^{-}	$SO_4^{=}$
Water analysis										
Well 1	7.82	4557	10.8	7.15	53.6	0.35	-	5.30	39.1	26.8
Well 2	7.66	8934	19.3	13.8	105.1	0.90	-	7.50	93.1	38.7
Soil analysis										
Soil	7.76	6195	4.6	3.2	88.3	0.67	-	4.95	65.7	26.1

Chemical Analyses

Malondialdehyde (MDA) content

The level of lipid peroxidation in barley leaves was quantified by determination of MDA, a breakdown product of lipid peroxidation according to Health and Packer (1968) and modified by Zaho *et al.* (1994).

Free amino acids

Free amino acids were determined according to Pellet and Young (1980) and Khan and Faiz (2008). From each fresh sample, 2g were extracted with 70% (*V/V*) ethanol. The ethanolic solution was filtered, concentrated and passed through a column cation exchange resin (Dowex 50H 100-200 Mesh). Elution was carried out with 70% (*V/V*) ethanol to take all carbohydrates, pigments and lipids present except free amino acids, then with ammonia solution 2M for elution of free amino acids. The previous steps were repeated again using HCl 0.01M instead of ammonia solution to complete elution of free amino acids. Each eluent was concentrated to a small volume by evaporation under vacuum at 50°C and kept deepfreezed until determined by physiological column for Sykam amino acid analyzer.

Quaternary ammonium compounds (glycinebetaine and choline)

Glycinebetaine and choline were determined by the method described previously by Grieve and Grattan (1983).

Electrophoretic pattern of soluble proteins

Soluble proteins in barley leaves were determined according to SDS-PAGE gel electrophoresis was performed in acrylamide slab gels following the system of Laemmli (1970) and as modified by Studier (1973).

Antioxidants isozymes

Superoxide dismutase (SOD) and catalase (CAT) were extracted from plant samples and separated by native polyacrylamide gel electrophoresis (PAGE) according to Weydert and Cullen (2010).

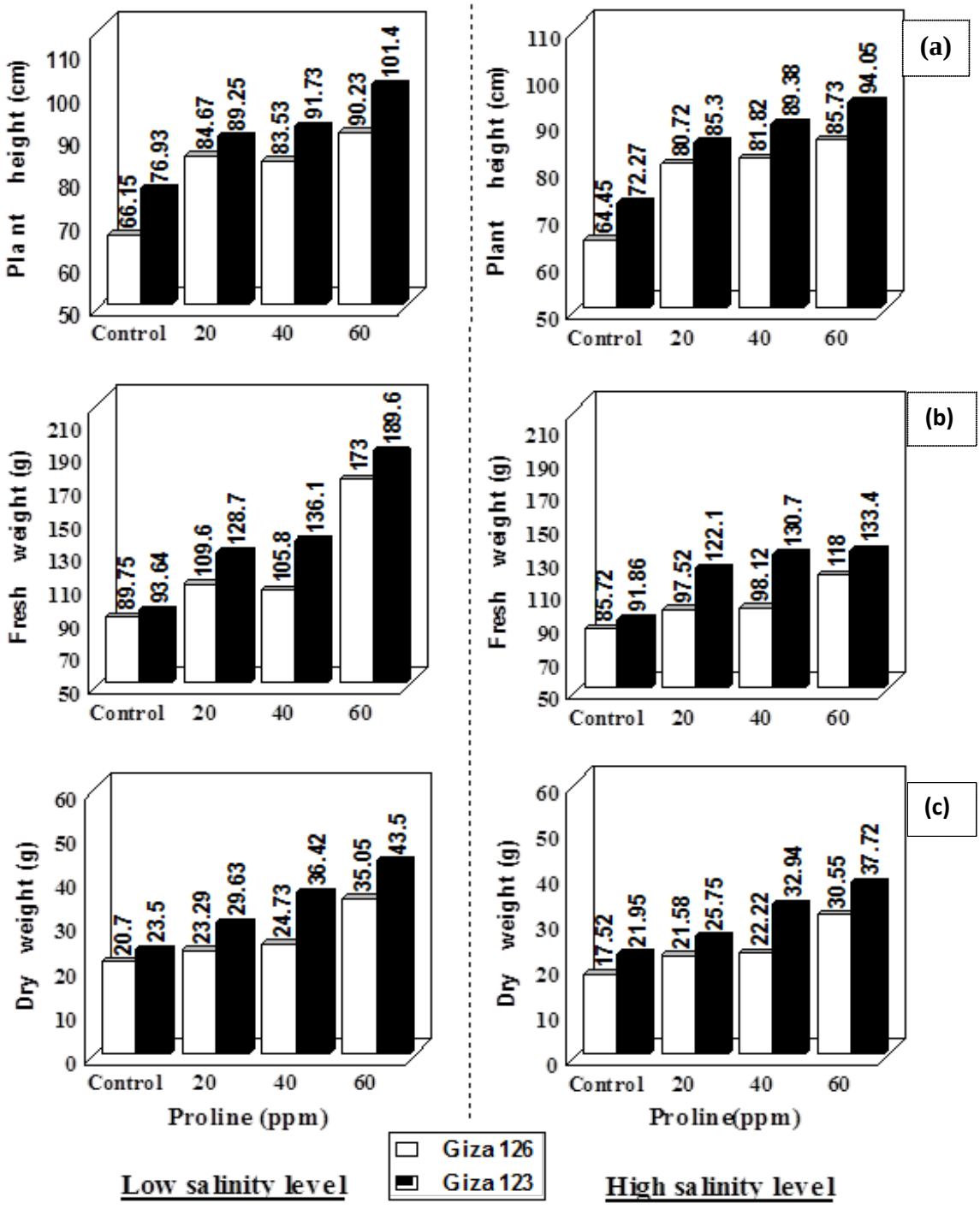
Statistical Analysis

Data were analyzed statistically according to the procedure outlined by (Snedecor and Cochran, 1982). Combined analysis over growing seasons was done when the homogeneity test was insignificant according to (Gomez and Gomez, 1984). Duncan's multiple range test was used for the comparison between means (Duncan, 1955).

RESULTS AND DISCUSSION

Plant Growth

Salinity stress caused a significant reduction in growth parameters *e.g.* plant height, fresh and dry weight of both barley cultivars (Fig. 1). However, exogenous application of proline counteracted the adverse effects of salinity on the growth of both barley cultivars. In this regard, improvement in growth parameters was much evident when proline applied at rate of 60 ppm under saline stress conditions. In this connection, Giza123 surpassed Giza126 at plant height, fresh weight and dry weight under two salinity levels. Also, the values of growth parameters under low salinity were more than



(a) Plant height (b) fresh weight (c) Dry weight

Fig. 1. Effect of foliar applications of proline on growth parameters of two barley cultivars under saline conditions

high salinity. These results were in complete harmony with those obtained by Ashraf and Foolad (2007) and Sadeghi (2011) on barley and Zaki and Radwan (2011) on wheat. It is worth mentioning that, effectiveness of proline applied as a foliar spray depends on the type of species, plant developmental stage, time of application and concentration (Ashraf and Foolad, 2007). In light of the positive effect of proline on growth parameters, Chandrashekar and Sandhyarani (1996), Hoque *et al*. (2007) and Ashraf and Foolad (2007) found that this was due to : 1) The important role of proline for protecting enzymes, three dimensional structures of proteins and organelle membranes. 2) Also supplies energy for growth and survival thereby helping the plant to salt tolerance.

Yield and its Components

Data presented in Fig. 2 show that, salinity affected negatively on yield and yield components (*i.e.*, plant height, biological yield, grain yield and straw yield) of two barley cultivars. On the other hand, foliar applications of proline significantly enhanced yield parameters compared with the control (without proline) under two salinity levels. The highest values of plant height, biological yield, grain yield and straw yield were recorded when proline applied at rate of 60 ppm. Comparison between the two cultivars data showed that the values of yield and its components in Giza123 were more than that in Giza126 under two salinity levels. While, the values of yield components under low salinity were higher than that under high salinity. These results were in complete harmony with that obtained by Abdel-Hameed (2004) and Zaki and Radwan (2011) on wheat. The reduction of yield and its components under saline stress conditions may be due to: 1) loss of spike-bearing tillers (Mass *et al.*, 1996). 2) Decrease in number of the filled grains/plant and 1000-grain weight (Dutt, 1988). 3) The osmotic inhibition of water absorption, the excessive accumulation of ions such as Na^+ or Cl^- in plant cells and/or in adequate uptake of essential nutrients (Munns and Termat, 1986).

Chemical Analyses

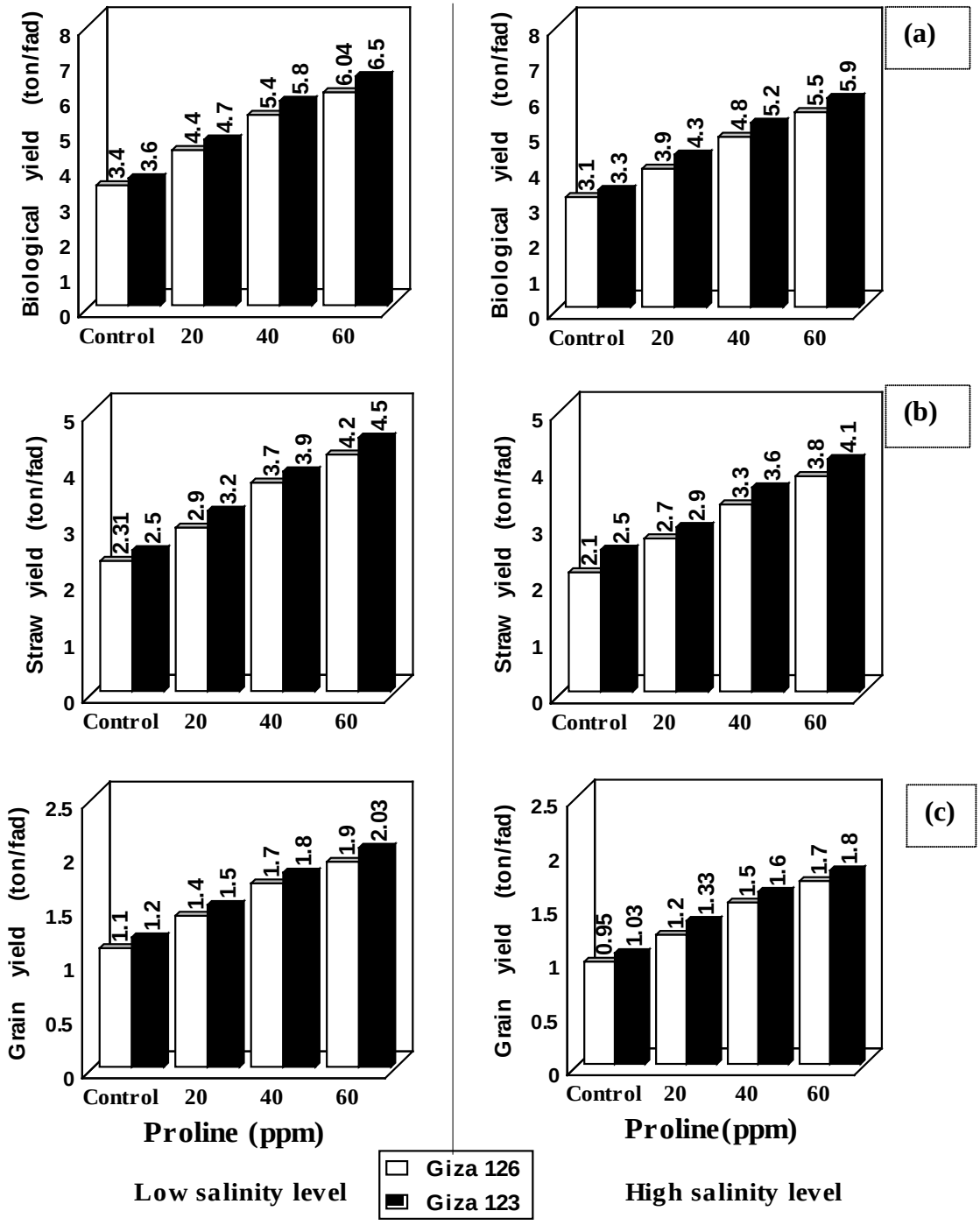
Malondialdehyde content

The level of lipid peroxidation in barley was quantified by determination of malondialdehyde (MDA). Malondialdehyde content in leaves of barley cultivars as affected by foliar applications of proline under saline stress are presented in Fig. 3. One of the biochemical changes possibly occurring barley plants are subjected to harmful saline stress conditions is the production of malondialdehyde and it was used as a biomarker to measure oxidative stress in barley. Salinity had a clear effect on the accumulation of malondialdehyde, but proline treatments led to a decline in malondialdehyde content (positive effect) compared with the control. The lowest value of MDA was recorded after treatment with proline at rate of 60ppm compared with the control. Also the accumulation of MDA under high salinity was higher than that accumulated under low salinity level. Regarding barley cultivars, Giza 126 accumulated MDA more than Giza 123 under two salinity levels. These results were in complete harmony with those obtained by Hendawey *et al*. (2014) on barley and Hendawey (2015) on wheat. In this regard, Borzouei *et al*. (2012) indicated that malondialdehyde content was higher in salt sensitive cultivar than salt tolerant cultivar.

Quaternary ammonium compounds (glycinebetaine and choline)

Data illustrated in Fig. 4 reveal that the effect of foliar applications of proline on accumulation of glycinebetaine and choline under saline stress in shoots of two barley cultivars. Salinity stress significantly increased glycinebetaine and choline accumulation in the two cultivars. However, the highest values from these compounds were obtained under high salinity compared with low salinity. Regarding the effect of proline treatments on accumulation of glycinebetaine and choline, data showed that the lowest values from glycinebetaine and choline were recorded at all doses of proline compared with control. Comparison between the two cultivars, Giza 126 significantly exceeded Giza 123 in glycinebetaine and choline contents under two salinity levels. The previous results were in agreement with those obtained by Chen *et al*. (2007) on barley, Nazarbeygi *et al.* (2011) on canola and Hendawey (2015) on wheat.

The accumulation of glycinebetaine in salt stressed plants has been proposed to play an important role in salt tolerance (Ashraf, 2004).



(a) Biological yield (b) Straw yield (c) Grain yield

Fig. 2. Effect of foliar applications of proline on yield parameters of two barley cultivars under saline conditions

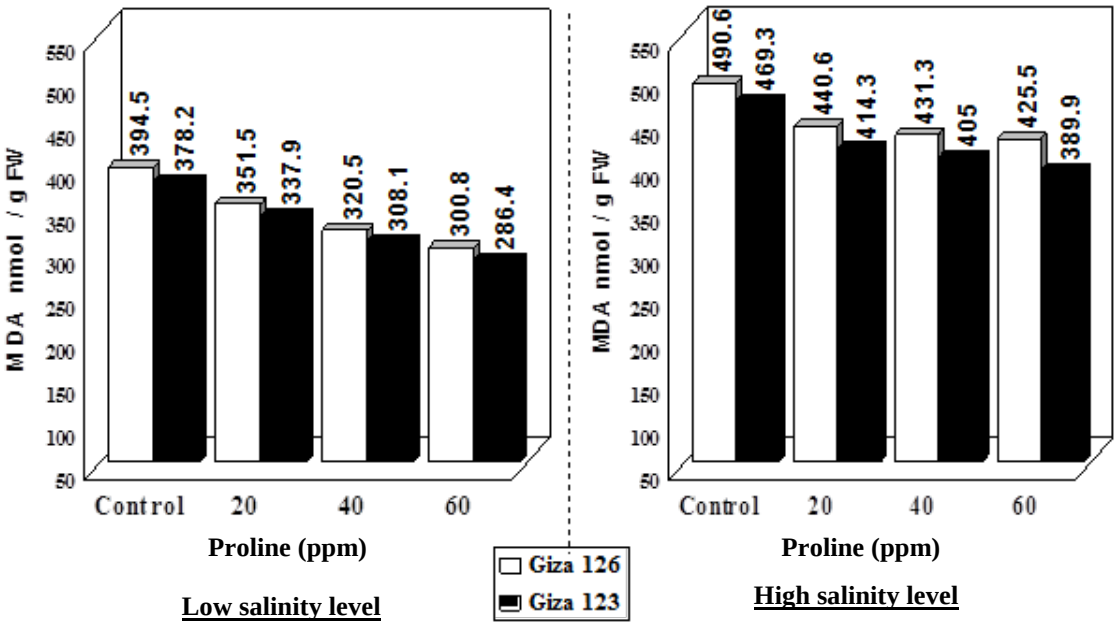
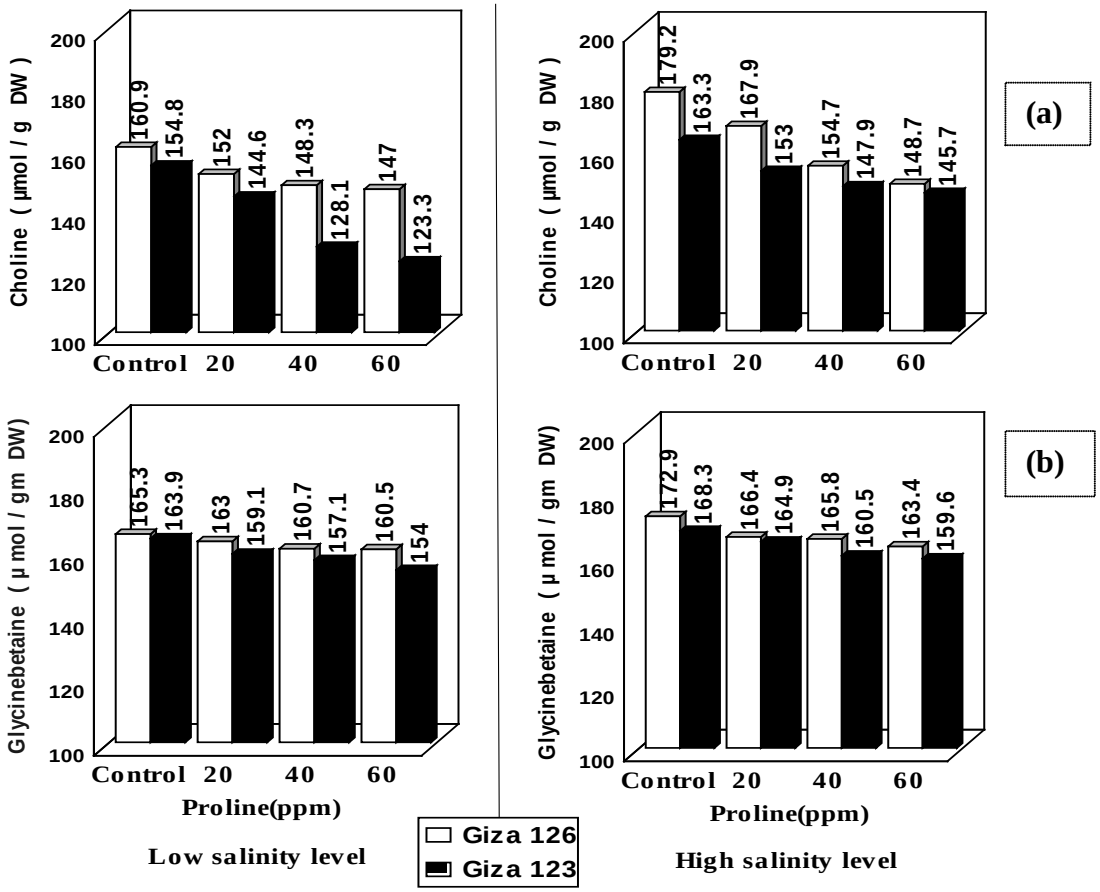


Fig. 3. Effect of foliar applications of proline on malondialdehyde content (ηmol/g FW) in shoots of two barley cultivars under saline conditions



(a) Choline (b) Glycine betaine

Fig. 4. Effect of proline foliar applications on quaternary ammonium compounds (µmol / g DW) content in shoots of two barley cultivars under saline conditions

The role of glycinebetaine in alleviating salt stress on plants may be due to: 1) It stabilizes both the quaternary structure of proteins and membranes (Sakamoto and Murata 2000), also stabilizing the structure of key proteins such as Rubisco (Makela *et al.*, 2000). 2) CO_2 assimilation rate increased (Yang and Lu, 2005), helpful in stabilizing pigments concentration (Cha-um *et al.*, 2007) and protecting the photosynthetic apparatus (Allakhverdiev *et al.*, 2003). 3) It ameliorates the harmful effects on gaseous exchange parameters (Kausar *et al.*, 2014). 4) It serves as compatible osmolytes, protectants of macromolecules and also as scavengers of ROS (Ashraf and Foolad, 2007) and preserving the osmotic balance (Gadallah, 2000). 5) It is related to the elevated SOD and CAT activity and alleviation of cell membrane damage by reducing oxidation of membrane lipid and improving the ion homeostasis (Hamdia and Shaddad, 2010).

Free amino acids

Data presented in Tables 2 and 3 show that free amino acids accumulation in two barley cultivars as affected by foliar application of proline under two salinity levels. However, the highest values of free amino acids were obtained under high level of salinity compared with the low level. On other hand, proline treatments had a positive effect on accumulation of free amino acids. In this connection, Hayat *et al.* (2012) summarized the important role of proline in plants under stress conditions in the following: 1) It protects the plants from various stresses and also helps plants to recover from stress more rapidly. 2) Enhanced growth and other physiological characteristics of plants. 3) Scavenges the ROS generated in plants under various biotic and abiotic stresses. 4) Affects plant-water relations by maintaining turgidity of cells under stress and also increases the rate of photosynthesis. 5) Protects plants from harmful stresses such as salinity. Also, Kavi Kishor *et al.* (2005) found that accumulation of proline was due to increased synthesis and decreased degradation under a variety of stress conditions. Data showed that thirty three amino acids were detected in the tested cultivars. The most abundant amino acids noticed were asparagines, proline, alanine, cystine, γ-aminobutyric and lysine where arranged according to the retention time of amino acids which separated from amino acid analyzer by physiological column. In addition, phosphoserine, α-minoadepic acid, α-aminobutyric acid, 1-methylhistidine and carnosine displayed low concentrations in two cultivars compared with the other free amino acids. Comparison between the two cultivars, data showed that Giza 126 exceeded Giza 123 in accumulation of free amino acids under two salinity levels. In this regard, other identified amino acids had concentrations between those extremes and different in their concentrations from cultivar to another and this depending on the interaction between foliar applications of proline and barley cultivars under saline conditions. In this regard, free amino acids play an important role to push the plants for salt tolerance. In this concern, Rai (2002) showed that plants subjected to stress, show accumulation of amino acids. The role played by accumulated amino acids in plants varies from acting as osmolyte, regulation of ion transport, modulating stomatal opening, and detoxification of heavy metals. Amino acids also affect synthesis and activity of some enzymes, gene expression, and redox-homeostasis. With respect to the accumulation of free amino acids under saline stress, there are many researches such as Ranieri *et al*. (1989), Roy-Macauley *et al*. (1992) and Mansour (2000), which showed that this accumulation is due to: 1) Inhibition of amino acids degradation. 2) Inhibition of protein synthesis. 3) Protein degradation. Also, Kavi Kishor *et al*. (2005) found that accumulation of proline was due to increased synthesis and decreased degradation under a variety of stress conditions.

Electrophoretic behavior of soluble proteins (SDS-PAGE)

The data presented in Tables 4 and 5 and Figs. 5 and 6 showed that number of bands in barley cultivars ranged from 12 to 22 with molecular weight ranged between 18 to 229 kDa under two salinity levels. However, under low salinity the more intensive bands are presented at molecular masses 18, 22 and 40 kDa for two barley cultivars. Bands of molecular masses 20, 25, 37, 56, 72 and 149 kDa were absent in two cultivars after treatment with all doses of proline and control. Also, band of molecular weight 229 kDa took the same trend (except the control of

Table 2. Effect of foliar applications of proline on free amino acids (mg/100 g FW) content in shoots of two barley cultivars under low salinity level

No.	Free amino acids	Treatments under low salinity level							
		Control		20 ppm		40 ppm		60 ppm	
		Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 26	Giza 123	Giza 126
1	**Phosphoserine**	0.003	0.019	0.078	0.006	0.003	0.010	0.549	0.004
2	**Taurine**	0.071	0.032	ND	0.062	0.038	0.012	0.026	0.009
3	**Phosphoethanol amine**	0.015	0.002	0.034	0.007	0.005	ND	0.103	0.002
4	**Asparaginic acid**	1.306	5.893	6.193	0.485	2.628	0.183	1.528	0.011
5	**Hydroxyproline**	0.414	0.707	0.262	0.396	0.042	0.629	0.547	ND
6	**Threonine**	3.747	4.655	4.395	1.794	1.621	0.748	3.791	0.028
7	**Serine**	4.462	4.256	5.186	3.025	1.859	0.460	3.754	ND
8	**Asparagine**	5.760	0.577	8.250	2.215	0.313	1.342	2.752	0.003
9	**Glutamine**	0.464	0.862	1.846	0.237	0.522	0.059	1.913	0.365
10	**α-Aminoadepic acid**	0.029	0.096	ND	ND	0.008	0.009	0.086	0.015
11	**Proline**	7.266	11.619	12.886	4.467	1.642	1.056	6.518	2.062
12	**Glycine**	3.472	2.603	26.234	2.249	1.724	7.967	3.369	0.397
13	**Alanine**	14.939	11.413	20.360	8.387	5.474	5.003	12.630	6.394
14	**Citrulline**	0.028	0.031	0.050	0.007	0.009	0.426	0.007	0.015
15	**α-Aminobutyric acid**	ND	ND	0.028	0.004	0.006	0.004	0.017	0.008
16	**Valine-6**	3.594	4.545	6.625	2.297	1.559	4.858	4.040	0.196
17	**Cystine**	5.860	14.938	10.910	3.402	4.630	0.301	7.448	2.027
18	**Methionine**	0.048	0.120	0.159	0.297	0.035	0.091	n.d	0.011
19	**Isoleucine**	2.396	2.077	3.570	1.310	0.605	1.730	1.844	0.004
20	**Leucine**	4.883	3.928	7.166	2.550	0.207	2.274	5.251	0.155
21	**Tyrosine**	3.516	1.178	5.539	2.271	0.093	0.886	4.811	0.074
22	**Phenyl alanine**	3.722	2.124	3.930	1.669	0.112	1.140	3.962	0.044
23	**β-Alanine**	2.220	0.149	0.610	0.722	0.153	0.191	1.656	0.175
24	**β-Aminobutyric acid**	2.304	0.016	0.614	1.183	0.014	0.059	1.831	0.023
25	**γ-Aminobutyric acid**	13.128	3.401	5.834	5.083	0.594	0.431	8.840	0.121
26	**Ornithine**	0.755	ND	0.059	0.623	ND	0.052	0.623	0.036
27	**Lysine**	19.706	10.900	6.495	15.843	ND	1.279	16.740	13.169
28	**3-Methylhistidine**	0.226	ND	0.046	0.178	ND	ND	0.464	ND
29	**Histidine**	0.643	0.198	0.122	0.230	ND	ND	0.689	ND
30	**1-Methylhistidine**	0.008	0.011	ND	ND	0.078	ND	ND	0.046
31	**Tryptophan**	0.050	0.013	0.078	0.013	ND	0.041	0.011	0.016
32	**Carnosine**	0.006	0.003	0.058	0.007	0.024	ND	0.013	0.020
33	**Argenine**	9.551	0.868	2.647	10.259	0.007	ND	9.580	7.019
Total free amino acids		114.59	87.23	140.27	71.28	24.01	31.24	105.39	32.45

Where: Amino acids in the table were arranged (Ascending) according to the retention time of amino acids which separated from column of amino acid analyzer apparatus. ND = Not detectable and FW= Fresh weight.

Table 3. Effect of foliar applications of proline on free amino acids (mg/100 g FW) content in shoots of two barley cultivars under high salinity level

No.	Free amino acids	Treatments under high salinity level							
		Control		20 ppm		40 ppm		60 ppm	
		Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126
1	**Phosphoserine**	0.019	ND	0.062	0.006	ND	0.007	0.004	0.005
2	**Taurine**	0.032	0.171	0.069	0.062	ND	0.018	0.133	0.095
3	**Phosphoethanol amine**	0.002	0.019	0.887	0.007	ND	0.120	0.047	0.004
4	**Asparaginic acid**	5.893	2.302	8.747	0.150	0.364	1.515	1.875	0.941
5	**Hydroxyproline**	0.707	n.d	0.635	0.104	0.068	1.123	5.202	0.333
6	**Threonine**	4.655	8.189	4.527	0.150	0.704	6.590	18.692	3.177
7	**Serine**	4.256	9.715	8.950	0.252	1.607	6.756	1.983	6.247
8	**Asparagine**	0.577	14.012	37.582	0.061	1.294	6.536	0.012	8.260
9	**Glutamine**	0.862	1.166	0.545	0.401	4.598	1.461	0.002	1.401
10	**α-Aminoadepic acid**	0.096	ND	ND	0.013	ND	ND	0.008	0.006
11	**Proline**	11.619	8.928	29.714	0.484	14.860	18.437	0.021	15.137
12	**Glycine**	2.603	8.384	8.552	0.178	3.322	5.332	0.002	3.592
13	**Alanine**	11.413	39.196	17.522	0.373	11.820	37.513	0.004	14.608
14	**Citrulline**	0.031	0.021	0.012	0.006	0.064	ND	0.006	0.045
15	**α-Aminobutyric acid**	ND	ND	0.008	0.004	ND	ND	0.006	0.003
16	**Valine**	4.545	8.998	5.742	1.491	4.443	8.889	7.128	4.210
17	**Cystine**	14.938	2.041	7.284	0.018	1.017	14.698	1.476	31.267
18	**Methionine**	0.120	0.152	0.212	0.049	ND	2.565	4.176	11.549
19	**Isoleucine**	2.077	4.266	2.583	0.732	2.052	5.719	0.002	3.273
20	**Leucine**	3.928	9.902	6.098	1.142	3.147	11.557	0.711	4.550
21	**Tyrosine**	1.178	3.817	3.854	1.267	0.923	9.454	1.294	10.151
22	**Phenyl alanine**	2.124	4.177	3.080	2.063	1.549	6.965	0.320	2.318
23	**β-Alanine**	0.149	0.219	0.807	0.183	ND	3.808	2.490	5.410
24	**β-Aminobutyric acid**	0.016	0.582	1.466	ND	0.270	3.819	2.699	6.290
25	**γ-Aminobutyric acid**	3.401	36.719	7.445	ND	4.717	19.146	7.936	10.144
26	**Ornithine**	ND	0.338	ND	0.228	ND	0.623	0.408	1.127
27	**Lysine**	10.900	23.248	9.007	27.367	5.430	27.964	15.619	25.331
28	**3-Methylhistidine**	ND	0.891	ND	0.064	ND	0.640	0.384	1.092
29	**Histidine**	0.198	0.839	ND	0.005	ND	1.491	0.305	0.259
30	**1-Methylhistidine**	0.011	ND	ND	ND	ND	0.030	0.004	0.003
31	**Tryptophan**	0.013	0.021	ND	0.470	ND	0.013	0.014	0.024
32	**Carnosine**	0.003	ND	ND	0.021	ND	0.145	0.009	0.005
33	**Argenine**	0.868	13.039	1.189	0.016	1.272	20.234	10.582	14.495
Total free amino acids		87.23	201.35	166.58	37.37	63.52	208.46	83.56	185.35

Where: Amino acids in the table were arranged according to the retention time (Ascending) of amino acids which separated from column of amino acid analyzer apparatus. ND = Not detectable and FW= Fresh weight

Giza 123). The same effect was observed with band of molecular mass 48 kDa (except the control of Giza 123 as well as 40 and 60 ppm). However, bands of molecular masses 28 and 59 kDa were absent in the control samples of two cultivars and after applying proline at rate 20 ppm (except 28 kDa for Giza 126 with 20 ppm). Also, polypeptides of molecular weights 85 and 66 kDa were absent from the control plants of Giza126 and proline treatment (20 ppm). Band of molecular weight 30 kDa appeared in Giza 123 after applied proline at all doses and control. While, it was absent in the control samples of Giza 126 and after applying proline at rates 40 and 60 ppm. Concerning band intensity, data showed that proline had a positive effect on band intensity under low salinity level. It was increased at molecular weights 40, 53, 97, 106 and 155 kDa for two cultivars after treatment with all doses of proline (except 40 and 97 kDa in samples of Giza 126 with 20 ppm) compared with the control. The same trend was true at 62 kDa when cultivars sprayed with proline at rates of 20 and 60 ppm. Also, Giza 123 showed increased in band intensity at 85 and 92 kDa when proline applied at all doses. There was increasing in band intensity at 22 and 34 kDa for two cultivars after treatment with 40 and 60 ppm (except Giza126 with 40 ppm) compared with the control. In the same direction, band at 30 kDa took the same trend when Giza123 treated with proline at rates of 40 and 60 ppm. Concerning band at molecular mass 45 kDa, it was increased in samples of Giza 126 and Giza123 after treatment with proline at 20 and 60 ppm, respectively. Also, barley cultivars showed increased in band intensity at 66 kDa by spraying proline at rates 40 and 60 ppm (except Giza123 with 60 ppm).In addition, band at molecular mass 92 was increased only in samples of Giza 126 when proline applied at rate 40 ppm compared with the control.

In the same direction, under high salinity the more intensive bands are presented at molecular masses 20, 34, 37 and 40 kDa for two barley cultivars. Band of molecular mass 229 kDa was absent in two cultivars after treatment with all doses of proline and control. Also, bands of molecular weights 22 and 28 kDa took the same trend (except the control of Giza 126 and treatment with proline at 20 ppm). The same effect was observed with bands of molecular masses 45 and 48 kDa in control and 20ppm but differ in 40 and 60 ppm where, the band of 45 kDa was absent in treatment with proline at 60 ppm and appeared at 40 ppm. In contrary band of molecular mass 48 kDa was presented in 6oppm and absent in 40 ppm. However, band of molecular mass 56 kDa were absent in the control samples of two cultivars and after applying proline at rate 20 and 60 ppm in Giza 123. Also, polypeptides of molecular weights 18 and 149 kDa took the same trend (except in Giza123 at the control and treatment with proline at 60 ppm). Concerning band intensity, data show that proline had a positive effect on band intensity under high salinity level. It was increased at molecular weights 34, 37 and 40 kDa for two cultivars after treatment with proline at 20 ppm) compared with the control. The same trend was true at molecular weight 20 kDa in all doses of proline (except 60 ppm) compared with the control. Bands of molecular weights 18 and 149 kDa were increased in band intensity at all doses of proline compared with the control. Also, Giza 123 showed increased in band intensity at 18, 20and 40 kDa (except 60ppm) when proline applied at all doses. There was increasing in band intensity at 22 and 53 kDa in Giza126 at all treatments of proline and control but at 25kDa except 40ppm. In the same direction, band at 34 kDa took the same trend when Giza126 treated with proline at all doses except 60 ppm. Comparison between the two levels of salinity, band of molecular weight 229 kDa was absent in control and foliar sprayed plants of Giza 126 in both salinity levels, however accumulated in Giza 123 only in control treatment under low salinity level. Bands of molecular mass 20, 25, 37, 56, 72 and 149 kDa were absent in all treatments under low salinity and accumulated in control and with all doses of proline in high salinity.

In general, the results in the previous tables and figures show that treatments had a clear effect on number and intensity of bands. Also, the number of bands was accumulated under high salinity level compared with the low level as well as the most bands had increased in intensity with increasing salinity level. It is worth mentioning that the accumulation of proteins at low molecular weights under high

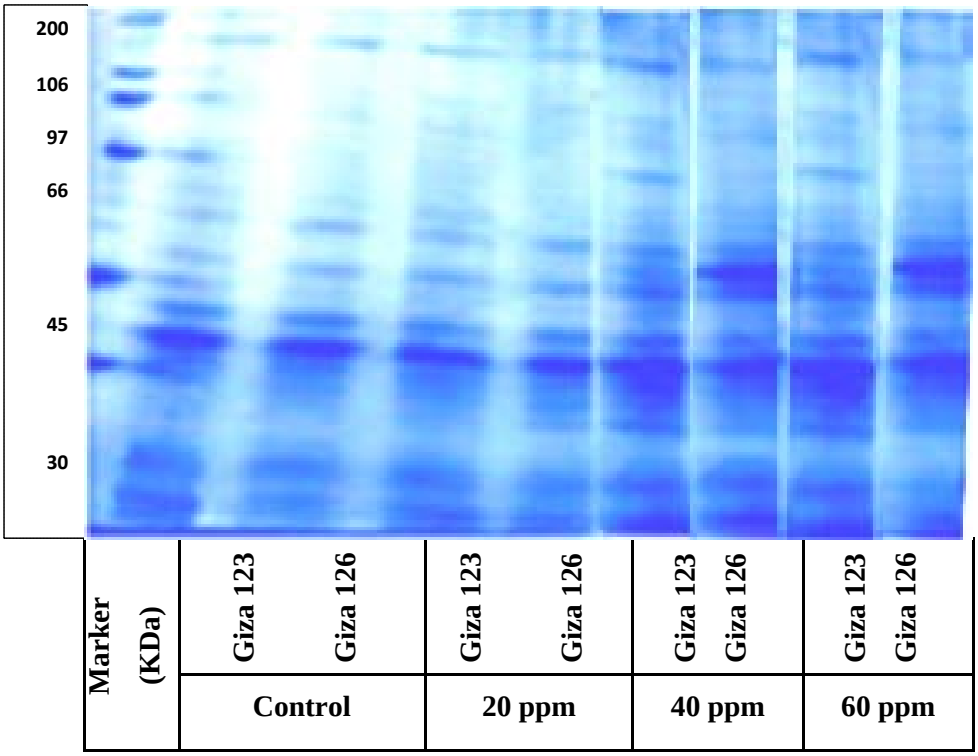


Fig. 5. SDS-PAGE patterns of soluble protein fraction extracted from fresh weight of Giza 123 and Giza 126 barley cultivars under low salinity level

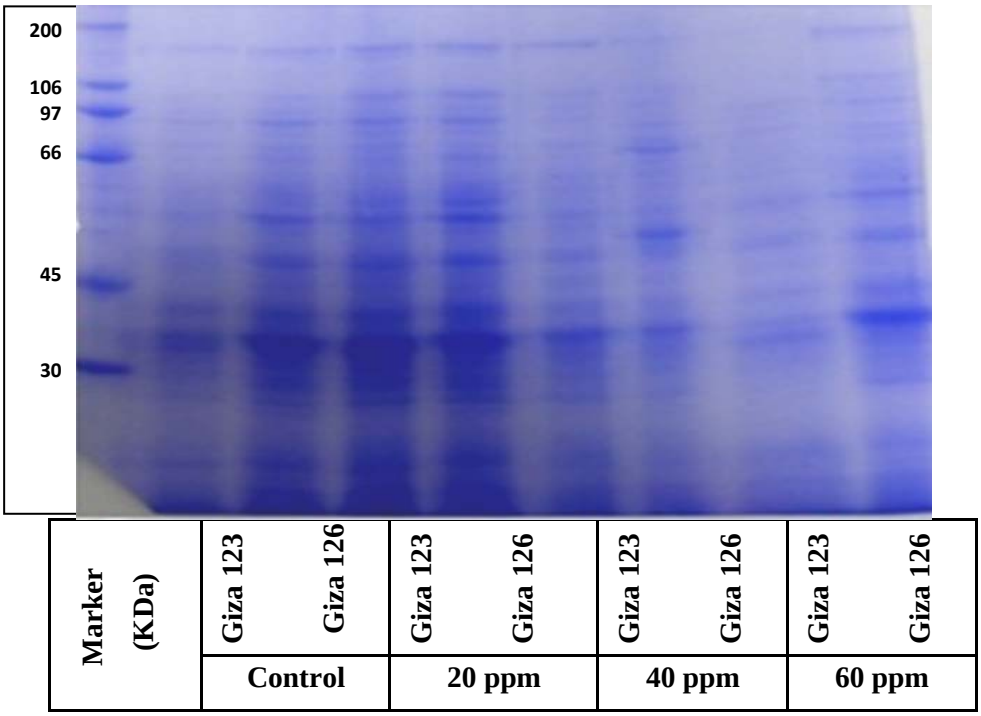


Fig. 6. SDS-PAGE patterns of soluble protein fraction extracted from fresh weight of Giza 123 and Giza 126 barley cultivars under high salinity level

Table 4. SDS-PAGE profile of soluble protein fraction extracted from fresh weight of Giza 123 and Giza 126 barley cultivars under low salinity

Band numbers	MW (KDa)	Treatments under low salinity level							
		Control		20 ppm		40 ppm		60 ppm	
		Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126
1	**229**	1.27	0	0	0	0	0	0	0
2	**155**	1.75	1.79	1.97	2.47	2.91	2.49	2.62	2.18
3	**149**	0	0	0	0	0	0	0	0
4	**106**	1.17	1.26	1.61	1.77	1.90	2.02	1.38	1.38
5	**97**	0.84	1.30	1.18	1.26	1.13	1.75	2.01	1.69
6	**92**	1.28	1.51	1.46	1.01	1.41	1.79	2.03	1.45
7	**85**	1.31	0	1.47	1.48	1.36	1.71	1.98	1.66
8	**72**	0	0	0	0	0	0	0	0
9	**66**	1.95	1.59	1.83	0	2.00	2.41	1.48	1.83
10	**62**	2.28	2.00	2.43	2.47	1.70	1.76	3.06	2.41
11	**59**	0	0	0	0	2.11	3.71	1.83	4.14
12	**56**	0	0	0	0	0	0	0	0
13	**53**	1.81	1.82	2.20	2.78	2.58	2.66	3.03	2.56
14	**48**	2.52	0	0	0	1.69	0	2.06	0
15	**45**	2.53	2.83	2.14	2.95	2.10	2.42	3.05	2.45
16	**40**	3.13	3.81	4.39	3.67	5.06	5.17	5.27	4.39
17	**37**	0	0	0	0	0	0	0	0
18	**34**	1.47	1.82	1.45	1.41	1.28	2.72	1.70	2.25
19	**30**	1.81	0	1.75	1.35	2.44	0	2.42	0
20	**28**	0	0	0	1.94	3.07	1.76	2.33	2.34
21	**25**	0	0	0	0	0	0	0	0
22	**22**	4.31	3.88	3.90	3.69	4.14	4.08	4.63	3.95
23	**20**	0	0	0	0	0	0	0	0
24	**18**	4.07	3.49	3.34	2.84	3.63	3.04	3.75	2.94
Total bands		16	12	14	14	17	15	17	15

Where; 0= no bands. 0.84= refers to the lowest band intensity. 5.27= refers to the highest band intensity.

Table 5. S-PAGE profile of soluble protein fraction extracted from fresh weight of Giza 123 and Giza 126 barley cultivars under high salinity

Band number	MW (KDa)	Treatments under high salinity level							
		Control		20 ppm		40 ppm		60 ppm	
		Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126
1	**229**	0	0	0	0	0	0	0	0
2	**155**	1.82	1.31	1.86	2.35	1.60	2.22	0	1.19
3	**149**	0	1.01	1.83	2.23	1.56	1.55	0	1.11
4	**106**	1.88	2.08	2.08	2.11	2.49	1.57	0	1.00
5	**97**	2.26	2.06	2.73	2.74	1.68	0	2.25	1.30
6	**92**	0	2.52	1.80	1.79	0	2.55	2.28	1.34
7	**85**	2.32	2.16	2.76	2.28	1.68	2.17	2.32	1.36
8	**72**	2.42	2.25	2.39	3.37	2.20	2.76	2.34	1.00
9	**66**	3.28	2.52	3.84	3.15	1.80	3.15	3.74	2.19
10	**62**	5.07	2.66	5.27	4.53	2.80	0	3.69	1.89
11	**59**	4.22	3.48	5.75	2.42	3.40	2.69	2.98	2.77
12	**56**	0	0	0	2.83	3.91	3.24	0	2.23
13	**53**	3.99	4.53	5.19	5.53	4.05	4.95	0	0
14	**48**	0	5.60	0	0	0	0	4.47	3.55
15	**45**	0	4.40	0	0	3.51	3.29	0	0
16	**40**	5.34	4.68	7.11	4.75	5.38	3.30	2.68	2.97
17	**37**	7.75	4.25	8.38	7.43	5.61	5.37	2.93	5.44
18	**34**	4.11	8.07	4.51	8.97	3.70	4.21	2.80	2.22
19	**30**	4.02	5.07	5.18	5.82	4.24	2.59	0	2.05
20	**28**	0	4.28	4.31	5.09	0	0	0	0
21	**25**	4.43	4.81	4.13	4.27	4.80	4.63	0	3.00
22	**22**	0	4.00	4.07	4.72	0	0	0	0
23	**20**	5.36	5.24	6.58	6.52	6.47	5.60	4.21	3.33
24	**18**	0	4.35	7.45	5.30	6.71	5.53	0	4.81
Total bands		15	22	20	21	19	18	12	19

Where: 0= no bands. 1.00= refers to the lowest band intensity. 9.07= refers to the highest band intensity.

salinity level may be related to the increase in synthesis certain sets of proteins (new bands) as molecular chaperons under saline stress. Molecular chaperones are a diverse group of proteins involved in various cellular functions comprising folding/unfolding, macromolecular assembly/ disassembly, keeping proteins in their native state and preventing their aggregation under various stress conditions, helping in protein synthesis/degradation and targeting to their cellular compartments (Boston *et al*., 1996). Of late they have been implicated in various physiological processes and plant defense under stress conditions (Chen and Shimamoto, 2011; Gupta and Tuteja, 2011; Hahn *et al*., 2011; Qi *et al*., 2011). Reddy *et al*. (2011). These results were closely related to results obtained by Abd El Rady (2009) and Hendawey and Hassany (2010).

Antioxidant Enzymes

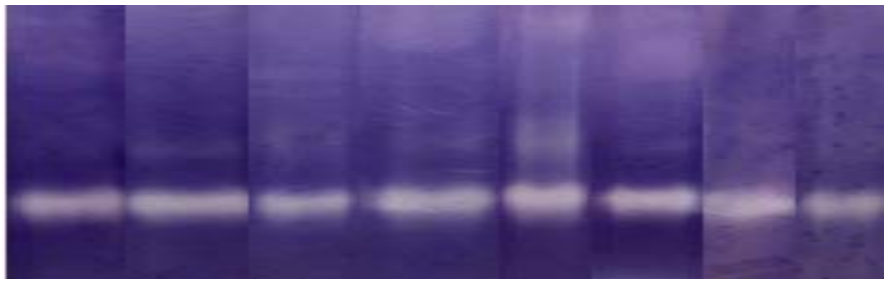
Superoxide dismutase (SOD) isozyme

Plant cells contain a large number of antioxidants to prevent or repair the damage caused by ROS, as well as to regulate redox-sensitive signaling pathways. SOD convert superoxide radical into hydrogen peroxide and molecular oxygen (Weydert and Cullen, 2010). In the present work, SOD patterns revealed the presence of about five bands for the two barley cultivars represented in Table 6 and Fig. 7. The more intensive band is presented at band number 4 in two barley cultivars with all doses of proline and control under two salinity levels. Bands number 1and 2 were absent in two cultivars after treatment with control and all doses of proline (except in Giza123 at 20ppm) under low salinity level. While, band number 1 was presented in both cultivars when proline applied at 20 and 40 ppm under high salinity level. Also, band number 2 was presented in Giza 123 and Giza 126 in case of control and absent at all doses of proline under high salinity level. Band (No. 3) was observed in two barley cultivars after treatment with proline at rate 20 and 40 ppm under two salinity levels. In same direction, band (3) not only disappeared at 60 ppm in two salinity levels but also in control samples of Giza 123 under low salinity level and Giza 126 under high salinity level. Also, treatments had a clear effect in absent of band number 5 (except Giza123 with 40 and 60ppm under high salinity level). Concerning band intensity, data showed that proline had a positive effect on band intensity under high salinity level. It was increased in all samples under high salinity compared with the low level. Band number 4 was increased in band intensity at 60ppm compared with the other treatments under two salinity levels. These results were agreed with those obtained by (Kolupaev *et al.*, 2005; Hendawey *et al*., 2010; Mahdi, 2011) on wheat. Expression of SODs genes are involved in many life aspects including developmental course and in response to environmental stress. Furthermore, SOD isoforms often respond differentially to various environmental stresses Mauro *et al*. (2005), indicating the importance of qualitative nature of SOD system in the scavenging of superoxide radicals Guan and Scandalios (1998). Hence, the identification of isozyme pattern of SOD and measuring the band intensity of each one is very important to investigate each isoform activity.

Catalase (CAT) isozyme

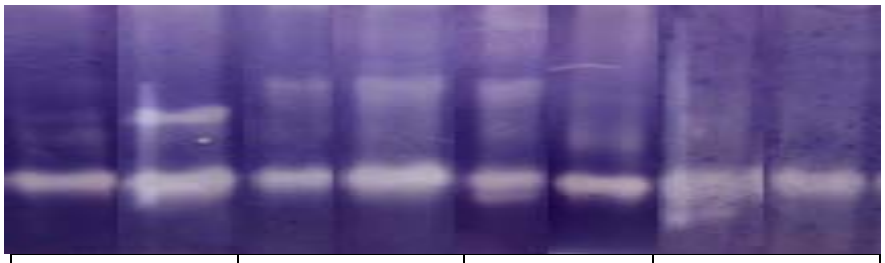
Catalase is one of the important enzymes that increase the antioxidant defense capability in plant cell under oxidative stress conditions, where it has an important role in the elimination of hydrogen peroxide in chloroplast, cytosol, mitochondria and peroxisome of plant cells Asada (2006). It is evident from the records in Table 7 and Fig. 8 that catalase patterns of studied barley genotypes revealed the presence of about five bands after treatment with proline. In this concern, the more intensive bands are presented at bands (No.4 and 5) in two barley cultivars at all treatments of proline and control under two salinity levels. Bands (No.1, 2 and 3) were absent in all treatments of proline and control under high salinity. On contrary, bands (No. 1 and 2) were detected in both cultivars after treatments of proline at rate of 40 and 60 ppm and not found in 20 ppm of proline and control under low salinity. In addition, band (No. 3) was appeared in proline treatment at 60 ppm and disappeared in other treatments and control under low salinity. Concerning band intensity, data showed that proline had a positive effect on band intensity under two salinity levels. Bands (No. 4 and 5) were exceeded in band intensity after treatments of proline at rate

(A) Super oxide dismutase under low salinity



Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126
Control		20 ppm		40 ppm		60 ppm	

(B) Super oxide dismutase under high salinity



Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126
Control		20 ppm		40 ppm		60 ppm	

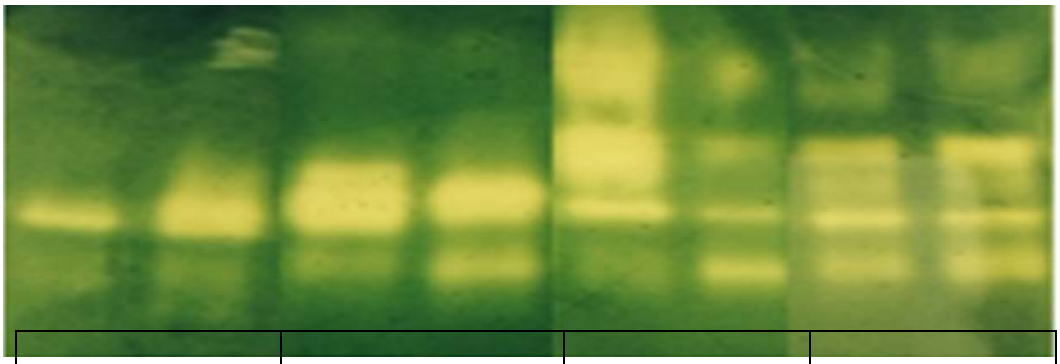
Fig. 7. A and B Zymograms of superoxide dismutase banding pattern isozyme in fresh weight of two barley cultivars as affected by proline foliar application under salinity stress

Table 6. Profile clarified superoxide dismutase isozyme pattern in fresh weight of two barley cultivars as affected by proline foliar application under salinity stress

Salinity level	Band number	Band intensity							
		Control		20 ppm		40 ppm		60 ppm	
		Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126
Low salinity	1	0	0	2.05	0	0	0	0	0
	2	0	0	2.58	0	0	0	0	0
	3	0	2.64	2.41	2.40	2.88	2.35	0	0
	4	8.03	8.37	7.80	8.44	8.56	8.06	9.18	8.12
	5	0	0	0	0	0	0	0	0
High salinity	1	0	0	4.12	4.66	5.51	2.78	0	0
	2	1.00	4.80	0	0	0	0	0	0
	3	1.46	0	3.15	3.62	3.57	3.78	0	0
	4	8.24	9.32	7.99	8.64	9.13	8.50	9.80	9.08
	5	0	0	0	0	3.11	0	3.88	0

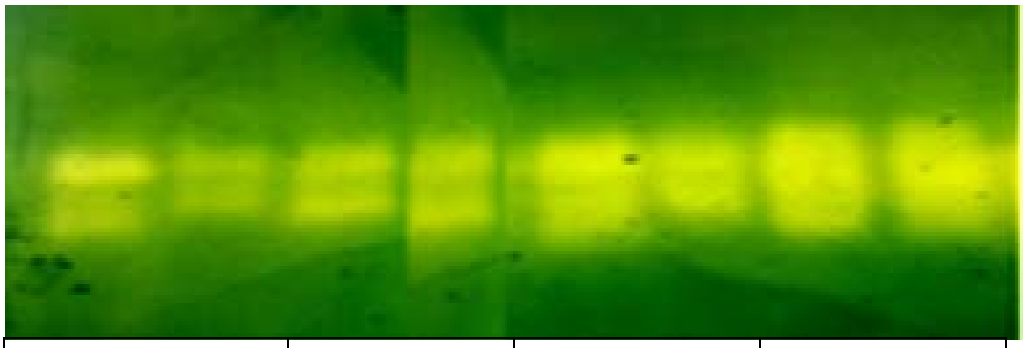
Where: 0= no bands. 1.00= refers to the lowest band intensity. 9.80= refers to the highest band intensity.

(A) Catalase under low salinity



Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126
Control		20 ppm		40 ppm		60 ppm	

(B) Catalase under high salinity



Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126
Control		20 ppm		40 ppm		60 ppm	

Fig. 8. A and B Zymograms of catalase banding pattern isozyme in fresh weight of two barley cultivars as affected by proline foliar application under salinity stress

Table 7. Profile clarified catalase isozyme pattern in fresh weight of two barley cultivars as affected by proline foliar application under salinity stress

Salinity level	Band number	Band intensity							
		Control		20 ppm		40 ppm		60 ppm	
		Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126	Giza 123	Giza 126
Low salinity	1	0	0	0	0	3.09	1.64	1.33	1.78
	2	0	0	0	0	4.18	1.82	1.90	2.35
	3	0	0	0	0	0	0	1.82	1.93
	4	2.02	2.94	3.91	3.00	1.62	0.62	1.48	1.75
	5	1.25	1.05	1.36	1.87	1.58	1.88	1.68	2.04
High salinity	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	4	2.37	1.00	1.79	1.92	2.47	1.99	2.96	2.89
	5	1.68	1.19	1.67	1.58	2.11	1.97	2.39	2.28

Where: 0= no bands. 1.00= refers to the lowest band intensity. 4.18= refers to the highest band intensity.

of 40 and 60 ppm under high salinity compared with the other level. While, the same bands were detectable changes in band intensity for both cultivars in treatment of proline at rate of 20 ppm and control under two salinity levels. Catalase enzyme is the main scavenger of strong oxidant H_2O_2 in peroxisomes and it converts hydrogen peroxide to water and molecular oxygen Willekens *et al*. (1995). In this regard, Bahari *et al.* (2013) showed that catalase activity increased by amino acids applications, also foliar spraying of amino acids can reduce the harmful effects of ROS and improves plant resistant under salt stress conditions. Generally, the increase of CAT activity is a strategy for improving salt tolerance Vaidyanathan *et al*. (2003).

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تراكم الأحماض الإيمينية ومساهمتها فى مقاومة الشعير للإجهاد الملحى تحت ظروف رأس سدر، جنوب سيناء، مصر

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أجريت تجربتين حقليتين خلال موسمى ٢٠١٢/٢٠١١ و٢٠١٣/٢٠١٢ بمحطة بحوث رأس سدر- جنوب سيناء- التابعة لمركز بحوث الصحراء وذلك لدراسة الدور الفعال للبرولين فى مقاومة الشعير للإجهاد الملحى، وتأثيره على صفات النمو والمحصول وبعض المكونات البيوكيميائية، ويمكن تلخيص النتائج كما يلى: البرولين كان له دور هام فى تخفيف الأثر الضار للملوحة على قياسات النمو والمحصول والمكونات البيوكيميائية فى صنفى الشعير (جيزة ١٢٣، جيزة ١٢٦)، كما أوضحت النتائج أيضا أن الصنف جيزة ١٢٣ كان أفضل تحت تأثير الملوحة مقارنة بالصنف جيزة ١٢٦، وفيما يتعلق بالأحماض الأمينية الحرة أوضحت النتائج ظهور ٣٣ حامض فى الصنفين وكانت الأحماض التالية (Asparagin, proline, Alanine, Cystine, γ-Aminobutyric acid, lysine) أعلى تركيزاً فى الصنفين، كما أظهرت النتائج أن معاملات البرولين أدت إلى إنخفاض فى قيم كل من Malondialdehyde ومركبات الأمونيوم الرباعية (الجليسين بيتان ، الكولين) مقارنة بالكنترول، كما أظهرت نتائج الإنزيمات المضادة للأكسدة وجود خمسة حزم لإنزيمى Superoxide dismutase ، Catalase فى صنفى الشعير قيد الدراسة (جيزة ١٢٦ ، جيزة ١٢٣)، وقد لوحظ أن كثافة الحزم band intensity تزيد تحت مستوى الملوحة العالى مقارنة بالمستوى المنخفض، وكان هذا واضحاً فى الصنف الحساس مقارنة بالصنف المقاوم، وقد أوضحت النتائج أن المعاملة بالبرولين وبخاصة ٦٠ جزء فى المليون كان لها تأثير إيجابى على الحزم مقارنة بالتركيزات الأخرى تحت مستوى الملوحة المنخفض، كما أظهر التفريد الكهربى للبروتينات الذائبة فى الصنفين محل الدراسة عن وجود ٢٤ حزمة مختلفة الوزن الجزيئي (18 - 229 KDa) تحت تركيزي الملوحة، وقد أوضحت النتائج غياب الأوزان الجزيئية (20, 25, 37, 56, 72, 149) تحت مستوى الملوحة المنخفض بينما غاب الوزن الجزيئي 229 KDa تحت مستوى الملوحة العالى مما يؤكد أن هذه الأوزان الجزيئية مميزة للملوحة العالية، ومن خلال هذه الدراسة يمكن الإستفادة من تقليل التأثير الضار للملوحة على أصناف الشعير باستخدام البرولين والذى يلعب دور هام فى دفع النباتات للمقاومة ضد الإجهاد الملحى.

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