



Effect of Proline on growth, yield, nutrient and amino acid contents of barley (*Hordeum vulgare* L.) irrigated with moderate saline water

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Abstract: Two field trials were carried out in the Research Farm of Faculty of Agriculture, Cairo University, Wady El-Notron Province, El Behaira Governorate, Egypt during two successive winter seasons of 2010-2011 and 2011-2012 to study the effect of different concentrations of proline on some growth parameters, yield and yield attributes and some chemical constituents of barley irrigated with moderate saline water under sandy calcareous soil conditions. The results showed that plant height, fresh and dry weight of plants/m², succulence and leaf area were significant positively affected by increasing the proline concentration from 0 to 100 μ mol. Moreover, 50 μ mol hemin concentrations recorded the highest values in most studied characters with significant differences as compared with control plant (zero hemin). Plant grown under salinity stress (zero hemin) contained greater concentrations of Ca, Na, Mn and Zn elements while, the hemin treatment up to 75 μ mol resulted in significant reductions of some macro and microelements. Such reduction was parallel to the increase in Mg up to 100 μ mol. Increasing hemin concentrations significantly increased all yield attributes and economic yield (kg/fed) as compared with control plant (0 hemin). The highest recorded values of number of spikelets/spike and 100 grain weight were recorded when 75 μ mol hemin was applied as compared with other concentrations. The results showed that the proline concentration was decreased nearly to the half of that occurred when barley plants treated with 100 μ mol of hemin indicating the mitigative and protective role of hemin in counteraction salinity of irrigation water. Generally we could conclude that, hemin act as plant growth regulators in alleviating harmful effects of salinity stress.

Key words: Barley, Salinity, proline, Growth, Yield, Mineral content, Amino acids.

Introduction

Agriculture is the backbone of most developing countries, because most of the population depending on it for their livelihood. Over the last few decades increasing food production in the conditions of an increasing world population is of a major concern. Several environmental factors (salinity, drought, nutrient imbalances, extreme temperatures, etc.) adversely affect growth, development, and final yield of plants. Salinity is one of the most serious abiotic stress factors that limit crop productivity. Salinity affects plant physiology at both the

whole plant and cellular levels by causing osmotic and ionic stresses. Salinity generates physiological drought, or osmotic stress, by affecting the water relations of the plant¹. osmotic stress and oxidative stress which lead to similar cellular adaptive responses, such as accumulation of compatible solutes, induction of stress proteins, and activation of reactive oxygen species (ROS) scavenging systems which comprise non-enzymatic and enzymatic antioxidants^{2,3}. A further common stress response in plants is the production of different types of compatible organic solutes⁴. In response to salinity stresses, plants adopt various strategies including modification in different physiological as well as biochemical processes such as signal perceptions and transduction cascades for survival against these stresses⁵. In parallel with traditional breeding and biotechnological strategies to improve the tolerance of plants to salinity⁶, several techniques have been proposed to improve plant performance in saline environments. These include seed or seedling priming⁷, and/or the application of stress metabolites that could be recognized and integrated by plants as components of a stress-induced adaptation response⁸. Foliar applications of osmo-protective molecules such as proline (Pro) have also been shown to have beneficial effects on plants exposed to salt stress⁹. The exogenous application of Pro enhances its endogenous level and therefore, promoting growth, antioxidant defense system and also decreases rate of uptake of Na⁺ and Cl⁻¹⁰. Several researchers showed that Pro alleviates negative impact on growth and development of crop and vegetable plants caused by different abiotic stresses¹¹. For instance, under salinity Pro reduces the accumulation of Na⁺ and Cl⁻¹², and in drought stress it facilitates water availability to the plant cell⁸. Applications of proline have been shown to improve yields in salt-stressed crops⁸, their effects on the quality parameters of commercial products are largely unknown. Nevertheless, the link between adaptation to stress and the accumulation of high-value metabolites may be important for agricultural production¹³.

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops in the world it is in the fourth position in total cereal production in the world after wheat, rice, and maize¹⁴. Barley is considered highly salt tolerant of the agriculturally important cereals and has been grown successfully in fields that irrigation has rendered unsuitable for other crops. Salt stress for barley at seedling stage has been mainly attributed to ionic effects rather than to osmotic effects¹⁵. The indication of good salinity tolerance at one growth stage such as germination and seedling does not necessarily mean that other stages will also have good salt tolerance. However, the levels of tolerance vary by the genotype and the developmental stage¹⁶.

Materials and Methods

Two field experiments were carried out at the Research Farm of Faculty of Agriculture, Cairo University, Waady El Notron Province, El Behaira Governorate, Egypt during two successive winter seasons of 2010-2011 and 2011-2012 to study the effect of different concentrations (0.0, 50, 75 and 100 mM) of proline on growth parameters, yield and yield attributes and some chemical constituents of vegetative and yielded grain barley under saline conditions. The soil texture of the experimental site was sand and some physical and chemical properties of a representative soil sample are listed in Table (1) according to the method described by¹⁷. Analysis of irrigation water is presented in Table (2).

Table 1. Mechanical and chemical analysis of the experimental soil site (Average of both seasons)

Character	Mechanical analysis				Chemical analysis											
					pH	EC dS/m	CaCO ₃	Organic Matter %	Soluble cations meq/l				Soluble anions meq/l			
	Sand %	Silt %	Clay %	Soil texture					Na ⁺	K ⁺	Mg ⁺⁺	Ca ⁺⁺	CO ⁻³	HCO ⁻³	Cl ⁻	SO ⁻⁴
Depth																
0 -30 cm	91	7.3	1.73	Sandy	8.00	0.5	12.25	0.15	0.83	0.28	3.62	2.61	--	1.36	3.52	2.46
30 - 60 cm	89.5	8.61	1.88	Sandy	8.20	0.7	10.52	0.23	0.86	0.35	2.95	2.77	--	1.5	3.39	2.04

Table 2: Chemical analysis of irrigation water

Characters	pH	EC dS/m	Soluble cations meq/l				Soluble anions meq/l			
			Na ⁺	K ⁺	Mg ⁺⁺	Ca ⁺⁺	CO ⁻³	HCO ⁻³	Cl ⁻	SO ⁻⁴
	7.80	5.00	26.30	0.71	4.45	13.20	0.02	4.52	35.10	5.02

The soil was ploughed twice, ridged and divided into plots. During seed preparation, 100 kg/fed calcium superphosphate (15.5% P₂O₅) and 50 kg/fed potassium sulphate (48 % K₂O) were applied. Nitrogen fertilizer with 60 kg N/fed as ammonium sulfate (20.6% N) was added in four equal doses began after three weeks and the other doses were applied weekly till flowering initiation. The proline treatments were sprayed at 30 and 45 days after sowing and allocate randomly in Randomized Complete Block Design (RCBD) with three replications. Each plot consisted of 15 rows (20 cm spacing) of 3.5 meter length, i.e. 10.5 m² (1/400 faddan), with seed rate of 40 kg/faddan. Planting date was 17th and 20th November in first and second seasons, respectively. Sprinkler irrigation took place immediately after sowing, then every one week intervals according to agronomic practices in the district.

Data recorded

Growth parameters: During both growing seasons, barley plants from each plot in 0.25 square meters were cut from ground surface at 60 days after sowing. Plant height (cm), total fresh and dry weight (g/0.25 m²) and water contents (%) were determined. Leaf area (cm²) was estimated according to the method described by¹⁸. The harvested shoots were then dried to constant weight at 70° and the values of succulence (ratio of fresh weight/dry weight) were calculated according to¹⁹.

Chemical composition of shoot: Plant samples were taken from each plot at 60 days after sowing, dried at 70°, and grounded using stainless steel equipments to determine K, Mg, Ca, Na, Fe, Mn and Zn concentration. Plant nutrients were determined as follows: Total nitrogen by using the micro kjeldahl method²⁰. Phosphorus, potassium and micronutrients were extracted by using dry ashing technique according to²¹. Phosphorus was photometrically determined using vanadate method and measured by spectrophotometer, while potassium was measured by flam photometer. Micronutrients and magnesium was measured using atomic absorption spectrophotometer.

Yield and yield components: At the harvest, one square meter from each plot was counted to determine number of spikes/m². Plant height (cm), spike length (cm), spike weight (g), spike grain weight (g), number of spikelets/spike and 100-grain weight were determined from randomly selected 20 tillers from each plot. The whole plot was harvested once and threshed to determine seed, straw and biological yields (ton/fed) as well as harvest index (grain yield/total biological yield) and crop index (grain yield/total straw yield) were calculated.

Nutritional value of grains: The dried grains were finally ground to K, Mg, Ca, Na, Fe, Mn and Zn concentration as mentioned by²¹. Total nitrogen percentage was determined according to the method described by²² and the crude protein content was calculated by multiplying total nitrogen concentration by factor of 5.75. Identification and determination of the amino acid composition of barely grains was carried out by using HPLC (Eppdraf, Germany) according to²³.

Statistical analysis: Data was analyzed using an analysis of variance of Randomized Complete Block Design²⁴. Since the trend was similar in both seasons, Bartlett's test was applied and the combined analysis of the two growing seasons was done. LSD (P < 0.05) was used to compare treatment means.

Results and Discussion

Growth parameters:

Data presented in Table (3) show that Exogenous applications of 50,75 and 100 mM of Proline (pro) significantly improved all growth parameters (plant height, fresh and dry weight of plants/m², succulence and leaf area) of salt-stressed barley plants. The effects of Pro on the growth parameters measured here were more

pronounced in 100 mM proline than the other concentrations and control plant. Among the most important factors responsible of retarded growth, under the influence of salinity, is the decline in photosynthetic performance²⁵. The data confirmed previous results reported by several authors with faba bean^{26,27} and common bean^{28,29}.

Table 3. Effect of proline concentration on some growth characters of barley plants irrigated with moderate saline water at 60 days after sowing. (Combined data of both seasons).

Character		Plant height (cm)	Weight (g/0.25 m ²)		Water contents (%)	Succulence	LA (ds m ²)
Treatment			Fresh	Dry			
Proline concentration (mM)	0.0	57.40	115.00	38.40	199.48	2.99	5.26
	50	59.00	170.00	56.00	203.57	3.04	9.38
	75	64.20	192.00	63.00	204.76	3.05	10.12
	100	70.80	198.00	64.00	209.38	3.09	10.70
F-Sig.		***	***	***	***	***	***
LSD _{5%}		4.53	5.50	4.35	29.27	0.29	1.15
CV (%)		5.73	2.37	6.25	9.43	6.73	10.35

***, F is significant different at 0.001% level, respectively, CV, Coefficient of variation

Thus, salinity usually causes a reduction in the leaf area which generally leads to a drastic reduction in net CO₂ assimilation³⁰. The increased thickness of the leaf under salinity stress may also reduce the photosynthetic performance via decrease of CO₂ diffusion in the mesophyll cells³¹. The osmotic effect resulting from soil salinity may cause disturbances in the water balance of the plant²⁵, including a reduction of turgor and on inhibition of growth, as well as stomatal closure and reduction of photosynthesis³². At the whole plant level the effect of stress is usually perceived as a decrease in photosynthesis and growth³³, and is associated with alteration in carbon and nitrogen metabolism³⁴.

Exogenous application of proline increased growth parameters under saline conditions as compared with the control treatment. It is probable that Pro would have been absorbed by the developing seedlings, where it maintained water status by increasing the influx of water and reducing the efflux of water under salt-induced water-limiting conditions³⁵. In fact, proline plays multifarious roles including adaptation, recovery, and signaling when it comes to combating stress in plants. Furthermore Pro might have protected cell membranes against ion toxicity and salt-induced oxidative stress, increased cellular growth³⁶, and thus increased the growth of common bean plantlets.

Chemical composition of shoot:

Data presented in Table (4) show significant differences between different concentrations of Proline on macro (Ca, Mg, K and Na) and micro (Fe, Mn and Zn) nutrients of barley shoot at 60 days after sowing. The untreated plant (zero proline) contained greater Na, Mn and Zn elements while, spraying plants with 100 mM proline gave the highest value of Ca, Mg, K and Fe compared to other treatments. Generally, shoot Na contents decreased gradually with increasing proline concentration from 0 to 100 mM.

Table 4. Effect of proline concentration on some chemical composition of barley shoot grown under saline condition at 60 days after sowing in 2011/12 season

Character		Macro-elements (Mg/100 g dry wt)					Micro-elements (ppm)			
Treatment		Mg (ppm)	Ca	K	Na	Ca/Na	K/Na	Fe	Mn	Zn
Proline concentration (mM)	0.0	3.95	0.55	1.33	2.50	0.22	0.53	0.44	0.98	0.05
	50	4.00	0.64	1.36	2.41	0.27	0.56	0.53	0.83	0.03
	75	4.50	0.89	1.55	2.11	0.42	0.73	0.71	0.83	0.03
	100	4.66	1.09	1.58	1.46	0.75	1.08	0.75	0.84	0.03
F-Sig.		**	***	ns	***	**	**	**	**	**
LSD _{5%}		0.28	0.13	0.21	0.32	0.06	0.21	0.15	0.02	0.01
CV %		3.23	8.04	7.28	7.53	8.45	14.41	12.05	1.48	14.63

ns, **,*** non significant and statistically different at 0.01 and 0.001 levels, respectively

The increases in the Na, Mg, Mn and Zn in the untreated plants are considered a normal result for increasing this element in the irrigation water (Table 4). Given that the dominant salt in saline soils is NaCl, both Na⁺ and Cl⁻ ions will occur in high concentrations. However, the contribution of Cl⁻ to growth reduction under salt stress is less well understood than that of Na⁺ in broad acre crops. This reflects the fact that most research on salt tolerance in cereals has focused on Na⁺ with little regard to Cl⁻ toxicity³⁷. Both Na⁺ and Cl⁻ should be given equal consideration since they are both metabolically toxic to plants if accumulated at high concentrations in the cytoplasm³⁸.

Exogenous applications of Proline significantly increased concentrations of K⁺, and the K⁺:Na⁺ ratio, and decreased Na⁺ ion levels in salt-affected plants. The results shown in Table 4 concur with data on faba bean²⁷ and soybean³⁹ and indicate that salt tolerance is associated with an enhanced K⁺:Na⁺ ratio. The ability of a plant to limit the transport of Na into its shoot is important to maintain a high growth rate and to protect metabolic processes from the toxic effects of Na⁺ ions⁴⁰. This could be attributed to the ability of roots to exclude Na⁺ from the xylem sap flowing to the shoot, which implies better growth of the shoot than the root⁴¹. The results here demonstrate that exogenous applications of Pro under saline stress conditions resulted in increased Ca, Mg, K and Fe levels and higher K⁺:Na⁺ ratios, but lower concentrations of Na⁺ (Table 4). Thus, Pro caused a reduction in Na⁺ ion absorption and toxicity. This could explain the mitigating effects of Pro on the growth of barley plants in saline soils. The antagonistic relationship between Na⁺ and K⁺ ions, as a result of Pro treatment, indicates that Pro could play a role in modifying K⁺:Na⁺ ratios under salt stress, which is reflected in reduced membrane damage and higher water contents under salinity stress. Accumulation of proline results in an increase in cellular osmolarity that drives influx of water or reduces its efflux thus providing pressure potential necessary for cell expansion⁴².

Yield attributes

Yield attributes (plant height, number of spikes/m², weight of spike, grains weight/spike and number of spikelets/spike), of barley plant grown under saline condition and affected by foliar treatment of proline Table 5. Data revealed that increasing proline concentrations significantly increased all yield attributes as compared with the untreated control. (Table 5). Maximum yield attributes were obtained in response to 100 mM as compared with control plant. The percent of increase was 21.12, 36.45, 23.96, 31.09, 28.79 and 11.07% for all the studied characters respectively compared with the control.

Table 5. Effect of proline concentration on barley yield attributes grown under saline condition at 60 days after sowing (combined data of both seasons)

Character		Plant height (cm)	Spike (s) character			Spikeletes no/spike	100-grain weight (g)
Treatment			Length (cm)	Weight (g)	Grains weight (g)		
Proline concentration (Mol)	0.0	64.40	10.70	1.92	1.46	13.20	11.20
	50	72.00	10.98	2.05	1.50	16.00	11.62
	75	74.00	12.00	2.35	1.90	16.80	11.66
	100	78.00	14.60	2.38	1.91	17.00	12.44
F-Sig.		***	***	***	**	***	***
LSD _{5%}		2.58	1.49	0.06	0.22	1.50	0.63
CV (%)		2.85	9.78	1.85	10.36	7.85	4.19

** ,*** F is statistically different at 0.01 and 0.001 levels, respectively

The reduction of yield attributes of untreated barley plants reveal the detrimental effect of moderate salinity of the irrigation water (Table 5). This also closely related to the decrease in growth parameters showed in table 3. The high mineral ion content of macro and micronutrient in control plants (0 proline) recorded in table 4 could be used as indicator to osmotic imbalance which reflected on the decrease in yield attributes in barley plants grown under salinity conditions. Salinity is one of the most serious stress factors that limit crop production. It can disrupt the plants' metabolic functions and can be easily noticed on the entire plant subsequently leading to decrease in productivity or even plant death. Moreover, the increase of sodium chloride and sodium sulfate concentration resulted in the reduction of number of tillers, length of spike, number of spikelets per spike, biomass per plant and grain yield per plant⁴³. The positive effect of proline on yield may be due to the vital effect of this amino acid stimulation on the growth and yield of plant cells. Our results are combatable with those obtained by⁴⁴ on *Pelargonium graveolens* L. and⁴⁵ on *Salvia farinacea* plants, they stated that application of amino acids led to the increments of flowering parameters and found that amino acids produced a high quality of inflorescences. The stimulatory effect were found to be correlated with the increase in content and activity levels of endogenous promoters particularly gibberellins and IAA which are known to promote linear growth of plant organs⁴⁶.

Yield (ton fed⁻¹):

Data presented in Table (6) represented the effect of increasing proline concentration on grain, straw and biological (ton/fed) as well as harvest and crop index of barley plant grown under saline condition. The data revealed significant increase on grain, straw and biological, when proline concentration increased from 0 to 100 mM as compared with the untreated control plant. Harvest and crop indexes increased with insignificant values by increasing proline concentration up to 100 mM.

Table 6. Effect of different proline concentration on grain, straw and biological yield/fed of barley grown under moderate saline conditions (combined data of both seasons)

Character		Yield (ton fed ⁻¹)			Harvest index (%)	Crop index (%)
Treatment		Biological	Grain	Straw		
Proline concentration (mM)	0.0	4.75	1.63	3.12	34.28	52.17
	50	4.83	1.70	3.13	35.13	54.16
	75	4.96	1.75	3.21	35.20	54.33
	100	5.23	1.84	3.39	35.09	54.07
F-Sig.		**	***	NS	***	***
LSD _{5%}		0.22	0.17	NS	3.43	9.09
CV (%)		3.35	7.82	6.45	7.85	13.34

ns, **, *** non significant and statistically different at 0.01 and 0.001 levels, respectively.

Reduction in seed yield of stressed plant might be attributed to the rapid reduction in leaf photosynthetic pigments and assimilates. Therefore, translocation of assimilates from stem to grains is the main source as well as limiting factors for growth and development of seeds. According to¹ salinity reduces plant productivity first by reducing plant growth during the phase of osmotic stress and subsequently by inducing leaf senescence during the phase of toxicity where excessive salt is accumulated in transpiring leaves. Soil salinity is a considerable problem adversely affecting physiological and metabolic processes, finally diminishing growth and yield⁴⁷. Recently,⁴⁸ concluded that salt treatment (100 mM of NaCl solution) depressed growth and yield production in 45 common and durum wheat varieties.

Proline can also protect cell membranes from salt-induced oxidative stress by enhancing activities of various antioxidants⁴⁹. For example, growth of tobacco suspension cells under salt stress was promoted by exogenous application of 10mM proline, which was proposed to be due to proline action as a protect ant of enzymes and membranes⁵⁰. In barley embryo cultures under saline conditions, exogenous application of proline resulted in a decrease in Na⁺ and Cl⁻ accumulations and an increase in growth⁵¹. Such ameliorative effects of proline were indicated to be due to plasma membrane stabilization⁵².

Chemical composition of yielded barley grains:

Macro and micro-elements:

Data presented in Table (7) illustrate the effect of increasing the proline concentration from 0 to 100 mM on some macro (Ca, Mg, K and Na), micro (Fe, Mn and Zn) nutrients and grain protein content. The data show insignificance effects on the most of the studied characters by increasing proline concentration except Mg, Na and Fe.

The low protein values of yielded barley grains grown under saline conditions reflect the inhibitory effect of salinity on plant growth, yield and its attributes (Tables 3,4,5). Consequently, concentration of protein was not clearly affected in the control plants. These results are confirmed the results obtained by⁴⁸ who found that the decrease in grain yield of wheat and durum might be caused by the salinity, which induced reduction of photosynthetic capacity leading to less starch synthesis and accumulation in the grain. In addition the results showed that salt accumulation increased protein content in five varieties and one accession of durum wheat. This variation maybe related to the relatively stable nitrogen metabolism under salt stress, which might contribute to the higher protein concentration.

Table 7. Effect of increasing hemin concentration on chemical composition of yielded barley grains grown under saline conditions in 2011/12 season

Character		Macro-nutrients (Mg/100 g dry wt.)						Micro-nutrients (ppm)			Total protein (%)
Treatment		Mg (ppm)	Ca	K	Na	Ca/Na	K/Na	Fe	Mn	Zn	
Proline concentration (mM)	0.0	2.67	0.15	0.37	0.95	0.16	0.39	0.24	0.79	0.04	13.10
	50	2.80	0.15	0.38	0.86	0.17	0.44	0.33	0.81	0.05	13.40
	75	2.83	0.16	0.39	0.82	0.20	0.48	0.35	0.81	0.05	13.65
	100	3.16	0.21	0.46	0.79	0.27	0.58	0.39	0.83	0.06	14.10
F-Sig.		***	***	*	**	*	ns	***	ns	*	ns
LSD _{5%}		0.12	0.01	0.06	0.07	0.02	0.04	0.04	0.10	0.01	0.71
CV (%)		2.09	5.71	7.36	3.90	6.18	4.50	5.76	6.31	8.88	2.63

ns, *, **, non significant and statistically different at 0.05, 0.01 and 0.001 levels, respectively.

It could be concluded that the stimulative effect of proline is through enhancing the biosynthesis of free amino acids and their incorporation into protein. These results supported by the results obtained by⁵³. The respective increase in inorganic ion contents (K⁺) at low and high concentration of proline is expected to be influenced by the effect of nitrogen compounds on protein synthesis, as proteins are required to transport protons, inorganic ions and organic solutes across the plasma membrane and tonoplast at rates sufficient to meet the needs of the cells⁵⁴. In addition, multiple membrane proteins may be needed for cations uptake from soil or solution to adopt varying extracellular conditions and nutrient availability⁵⁵. Positively charged macronutrients such as potassium (K⁺) are required in relatively large amount for plant growth and development. Thus, the above mentioned results are consistent with the results of growth parameters (Table 2). This means that amino acid (proline) influence the absorption and transport of cations, as reported by⁵⁶. proline led to increase in the contents of ions in the main organs of the wheat plant through their role in regulating various processes including absorption of nutrients from soil solution⁵⁷.

Amino acid contents:

It has been found in the present investigation that, barley plant grown under saline conditions and sprayed with different concentrations of proline gave higher amino acids, most of essential amino acids and total amino acids as compared with control. Also foliar treatment increased the contents of total amino acids, essential amino acids and the ratio of essential to non-essential amino acids compared with control plants. These obtained results are in harmony with those obtained by⁵⁸ who reported that, amino acid treatment enhanced the levels of total amino acids, essential amino acids and the ratio of essential to non-essential amino acids in wheat plant.

Table 8. Effect of different proline concentrations on Amino acid contents (g/100g dry weight) barley grains grown under saline conditions in 2011/2012 season.

Treatment Amino acids	Proline concentrations (mM)			
	Control	50	75	100
Aspartic	0.47	0.52	0.39	0.52
*Threonine	0.23	0.26	0.27	0.22
Serine	0.42	0.33	0.43	0.34
Glutamic A	2.54	2.19	2.45	2.72
Proline	0.04	0.07	0.16	0.07
Glycine	0.60	0.60	0.78	0.66
Alanine	0.54	0.60	0.53	0.60
*Valine	0.56	0.53	0.48	0.55
*Isoleucine	0.33	0.27	0.27	0.29
*Leucine	0.68	0.66	0.75	0.80
*Phenylalanine	0.34	0.27	0.31	0.34
*Histidine	0.20	0.16	0.10	0.20
*Lysine	0.25	0.22	0.18	0.29
Ammonia	0.62	0.71	1.17	0.52
*Arginine	6.18	6.61	6.74	5.87
*Essential	8.53	8.72	8.83	8.35
Non-essential	4.85	4.57	5.00	5.14
Total amino acid	13.38	13.29	13.83	13.49
Ess./Non-Ess.	1.76	1.91	1.76	1.63

These results are in a good harmony with those obtained by⁵⁹. They concluded that, metabolic acclimation via the accumulation of compatible solutes is often regarded as a basic strategy for the protection and survival of plants under abiotic stress. Many plant species accumulate significant amounts of glycine betaine, proline, and polyols in response to high salinity⁶⁰. Stress occurs from two ways due to increase of proline, which includes increasing synthesis of proline enzymes levels and decreasing of destructive proline enzymes actions⁶¹. Recently, it was also shown that some of these compatible solutes are very efficient in reducing the extent of K⁺ loss in response to both salinity⁶² and oxidative stress⁶³ in barley.

Conclusion

Barley plants grown under saline condition exhibited several behaviors which imitate the deleterious effect of salinity on plant growth, macro and micronutrient, yield attributes and economic yields and nutritional values of produced grains. External application of different proline concentrations showed positive result on all studied parameters. Barley plants treated with proline showed mitigative and protective role in counteraction salinity of irrigation water. So, proline application is a promising in alleviating salinity stress effects.

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