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Effect of salt stress on plant growth and physiological parameters of common glasswort (*Salicornia europaea*)

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Abstract

Salinity is one of the most serious environmental problems influencing crop growth *Salicornia* spp. is one of the most salt tolerant plants. The salinity-growth response curve for *S. europaea was* evaluated in a hydroponic study in the growth chamber of the field crops department, faculty of agriculture, Ege University, Turkey through April to September 2014. The main objective was to determine the effects of salt stress on *S. europaea*. The factors were salinity stress (0,100, 200,300,400 and 500 mM NaCl in the root medium).The experimental design was a factorial experiment in the base of randomized plot design with three replications. The results revealed that *S. europaea* tolerated salinity up to 500 mM NaCl; however, the optimum growth was at 200mM NaCl in the root medium. At 100 mM NaCl *S. europaea* recorded the highest fresh weight per plant (124.33 g/plant). However, at 200 mM NaCl *Salicornia* showed the highest shoots length (38cm), the tallest root (45cm), and the highest number of lateral branches (61.67). The treatments 100 and 200 mM NaCl recorded the highest stem diameter (2.24mm), and the tallest node (11mm). The superoxidismutase (SOD) and peroxidase (POD) activities, protein and proline (PRO)contents were increased as a result of salinity stress, where 500 mM NaCl recorded the maximum values: 29.21 µ mol min^{-1} mg^{-1} protein, 60.61 U mg^{-1} protein, 0.14%, and 1.27 µmoles/g FW respectively. The catalase (CAT) activity was significantly increased at 200 mM NaCl, but at higher levels of NaCl, i.e., 300, 400 and 500 mM, CAT activity significantly decreased in comparison with control plants. Based on the data obtained, it is clear that the halophytic characteristics of *Salicornia europaea* plants as evidenced by the positive effect of moderate salinities on plant growth.

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Introduction

Salinity is one of the most serious environmental problems influencing crop growth (Lopez *et al.*, 2002). About 23% of the world's cultivated lands are saline (Khan and Duke, 2001). Soil salinity refers to the presence of high concentration of soluble salts in the soil moisture in the root zone. This high concentration of salts interferes with balanced absorption of essential nutritional ions by plants (Tester and Devenport, 2003). Increasing soil salinization and the growing scarcity of fresh water dictate the need for a creative solution to attain sustainable crop production (Ventura and Sagi, 2013). There are several approaches to deal with these salt affected soils such as scraping, flushing and leaching. However, these methods were found to be very expensive and consume large quantities of fresh water (Jouyban, 2012). Therefore, attention was given to use alternative techniques, such as seawater agriculture through utilization salt tolerant plants (halophytes) as potential cash-crops (Khan and Weber, 2006). The utilization of halophytes is cheaper and more economical in the reclamation of saline soils (Ashour *et al.*, 2002). *Salicornia* (sea-beans or drift seeds) (Lu *et al.*, 2010) which belongs to the Chenopodiaceae family (Ghaffari *et al.*, 2006) has emerged as oilseed halophyte for seawater irrigation (Glenn *et al.*, 1991). It is one of the most salt tolerant plants generally, capable of growing under hyper-saline drainage water (Grattan *et al.*, 2008). Moreover, it is a promising resource to cultivate under extreme climatic conditions of arid-desert regions (Rueda-Puente *et al.*, 2013). *Salicornia* is an annual plant growing to 30 cm. It is flowering from August to September, and the seeds ripen from Sep to October (UK-Plants for a Future, 2014). *Salicornia* has good potential as both forage (Swingle *et al.*, 1996) and an oilseed crop. Under natural growth conditions, the seeds contain about 30% oil and about 35% protein (Bashan *et al.*, 2000). The oil is polyunsaturated and similar to sunflower oil in its fatty acid composition. The expected yield is 1700 kg plant biomass 1000 m^{-2} land and 200 kg seeds1000 m^{-2} land, a seed yield similar to that of soybean and sunflower (Glenn *et al.*, 1998). On the other hand, *Salicornia* contained high vitamins and minerals, which made it an ideal nutritional and dietary supplement (Lu *et al.*, 2010). The oil is high in linoleic acid (80%) and less oleic acid 12% (Anwar *et al.*, 2002). The seed oil could replace soybean oil in chicken diets (Glenn *et al.*, 1991). Also, many parameters of *Salicornia* seed oil were quite compatible with those of safflower oil (Anwar *et al.*, 2002). After oil extraction, the remaining high-protein meal (43% protein) is fed to animals (Glenn *et al.*, 1991). In addition, young shoots of *Salicornia* can be eaten as a vegetable and are currently marketed in Europe (Clark, 1994). The shoots are rich in the lipophilic antioxidant beta-carotene, with 15.9 mg 100 g^{-1} fresh weight, which makes the plant a good source of vitamin A (Lu *et al.*, 2010). Up to now, *Salicornia* has been successfully cultivated in different countries such as Mexico, India, Eritrea, Saudi Arabia and the United Arab Emirates (Milan and Stanislav, 2002). It has been reported that common glasswort (*Salicornia europaea* L.) can survive up to 1,020 mM NaCl with no phytotoxic effect, for growth ranging from 136 to 510 mM NaCl (Macke and Ungar 1971). Other glasswort species, such as dwarf saltwort (*Salicornia bigelovii* Torr.) showed normal growth at 200 mM NaCl (Ayala and O'Leary ,1995).Therefore, the main objective of this study was to determine the effects of salt stress on some morphological and physiological parameters of the *S. europaea* under greenhouse conditions.

Materials and methods

Plant material and growth conditions

This study was carried out at EGE University, Izmir, Turkey through April to September 2014. S. *europaea* seeds were sown on peat soil and grown in a growth chamber under a 16:8 light: dark photoperiod, 15/24°C night/day temperature and 70% humidity. After two months, the seedlings were washed free of soil before being transplanted individually in plastic pots with the silica sand bed and containing (in mM): K^+, 3.001; Ca^{2+}, 2; Mg^{2+}, 0.5; NO^{3-}, 5; NH^{4+}, 1.001; HPO_4^{2-}, 1;SO_4, 0.516; Cl^-, 0.001; H_2BO^{3-}, 1210.025; Mn^{2+}, 0.002; Zn^{2+}, 0.002; Cu^{2+}, 0.001; Mo^{2+}, 0.001; Fe-Na-EDTA, 0.01, buffered with 2 mM MES, (pH

6.0). Seedlings were grown for 15 days to recover from transplanting; over these 14d, the plants resumed growth as demonstrated by an increase in length of the main stem. During the recovery period, no NaCl was added to the nutrient solution and seedlings were covered with transparent plastic pots to keep the relative humidity of the air above 90%. After the recovery period salt treatments were started. Plants were randomly allocated into 6 treatment groups, consisting of 0 (control), 100, 200, 300, 400, 500 mM of NaCl. The experimental design was a factorial experiment in the base of randomized complete design with three replications. After two months of salt treatments, the plants were harvested, measured and analyzed. Shoot length (cm), Root length (cm), Number of lateral branches, Stem diameter (mm), node length (mm), and fresh weight (g/plant) were immediately determined after harvest.

Chemical analysis

After weighting, fresh plant material was divided into two parts. One part was frozen in liquid nitrogen and stored at −80°C for later use. The remaining fresh plant material was dried at 70°C for 24 hours, and the dry weight was measured. The frozen parts were used for free proline content which was determined according to Bates *et al.* (1973), and for enzyme extracts according to Edreva and Cholakova (1975). The peroxidase activity was measured according to Herzog and Fahimi (1973). Total SOD activities were determined by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) according to the protocol of Beauchamp and Fridovich (1971). One unit of SOD activities was defined as the amount of enzyme required to cause 50% inhibition of reduction of NBT as monitored at 560 nm and the activities were expressed as units per milligram of protein. Total CAT activities were assayed by measuring the initial rate of disappearance of H_2O_2 according to Bergmeyer (1970), the decrease in absorption was followed for 30 s and CAT activities were expressed as units (µmol of H_2O_2 decomposed per minute) per milligram of protein. However, protein content of the samples was determined by the Bradford method. Bovine serum albumin was used as a standard (Bradford, 1976).

Statistical analysis

The data were statistically analyzed and the significant means were compared by Duncan's multiple range test (DMRT) at 5% probability level using the software MSTATC and MSExcel.

Results and discussion

Effect of salinity stress on plant growth

Effect of salinity on shoot length, root length, and lateral branch number

The present investigation indicates the halophytic characteristics of *Salicornia* plants as evidenced by the positive effect of moderate salinities (200 mM NaCl) on plant growth (Fig. 1). The responses of *Salicornia europaea* to salinity stress are shown in Table 1. The results indicate that there was a gradual increase in shoot length, root length, and lateral branch number with increasing the concentrations of salts until it reached the maximum at a concentration of 200 mM NaCl. Then a gradual decline in growth happened until it reached the lowest at a concentration of 500 mM NaCl for shoot length and lateral branch number, and at a concentration of 0 mM NaCl for root length. On the other hand, the 200 mM NaCl recorded the tallest plants (38cm), tallest root (45cm), and highest lateral branch number (61.7); however, the salt treatment 500 mM NaCl showed the shortest plants (27cm), and the lowest lateral branch number (36). Similar to our results, Ayala and O'Leary (1995) reported that *S. bigelovii* showed a lower plant height in 5 vs. 200 mM NaCl and they attributed the reduced plant growth to toxic effects after hyper accumulation of K^+, Ca^{2+}, and Mg^{2+} by shoots to compensate for sodium deficiency. Park *et al.* (2013) recorded that based on the shoot length; the growth rate of common *S. europaea* was increased more at 300 mM NaCl than it did at 0 and 700 mM NaCl.

Katschnig *et al.* (2012) found that the optimal growth for *S. dolichostachya* occurred at 300 mM NaCl, and growth declined when concentrations were further increased to 500 mM NaCl. Aghaleh *et al.* (2009)

reported that, the shoot growth of *S. persica* and *S. europaea* species were increased under low NaCl concentration (100 mM) and then decreased with increasing NaCl concentrations. In contrast to *S. persica*, root length in *S. europaea* reduced steadily with an increase in salinity. On the other hand, Ungar (1978), showed that lateral branch number of *Salicornia europaea* was increased with increasing NaCl treatments until it reaches the maximum at 170mM NaCl, then decreased until it reaches the minimum at 510 mM NaCl. As stated by Munns (2002), suppression of plant growth under saline conditions may either be due to decreased availability of water or to the toxicity of sodium chloride.

Table 1. Effect of salinity stress on shoot length (cm), root length (cm), and lateral branches number of common glasswort (*Salicornia europaea*).

Treatments	NaCl	Shoot length (cm)			Root length (cm)			Lateral branches (no)		
	(mM)	M		SD	M		SD	M		SD
1	0	28.67	±	1.5	19.00	±	2.0	49.67	±	6.4
2	100	32.33	±	2.3	40.67	±	2.1	59.67	±	8.0
3	200	38.00	±	4.4	45.00	±	7.0	61.67	±	14.6
4	300	36.00	±	2.0	37.00	±	4.4	54.33	±	5.1
5	400	28.67	±	1.5	34.67	±	4.6	43.33	±	6.1
6	500	27.00	±	1.0	22.33	±	4.9	36.00	±	1.0

*The data represent mean ± SD of three replicates, **M= Mean, SD= Standard deviation.

Table 2. Effect of salinity stress on fresh weight (g/plant), stem diameter (mm) and node length (mm) of common glasswort (*Salicornia europaea*).

Treatments	NaCl (mM)	Fresh weight (g/plant)			Stem Diameter (mm)			Node Length (mm)		
		M		SD	M		SD	M		SD
1	0	47.23	±	7.3	2.20	±	0.01	6.00	±	0.0
2	100	124.33	±	21.1	2.24	±	0.01	11.00	±	1.0
3	200	116.00	±	17.3	2.24	±	0.00	11.00	±	1.0
4	300	97.67	±	15.3	2.22	±	0.01	8.67	±	0.6
5	400	77.00	±	11.8	2.22	±	0.01	7.00	±	0.0
6	500	71.00	±	10.4	2.21	±	0.00	7.00	±	1.0

*The data represent mean ± SD of three replicates, **M= Mean, SD= Standard deviation.

Effect of salinity on fresh weight, stem diameter and node length

Data in table 2 showed that, the same trend was observed with fresh weight, stem diameter and node length of *S. europaea,* which were increased with increasing salinity stress until it reached the maximum at concentrations of 100 and 200 mM NaCl and then gradually decreased until it reached the very least, with concentration of 500 mM NaCl. These findings are in agreement with Aghaleh *et al.* (2011), who found that, the fresh weights of two *Salicornia* species (*S. persica* and *S. europaea)* were increased significantly at 85 and 170 mM NaCl and decreased at higher concentrations. Khan *et al.* (2001) also reported that the optimal shoot fresh weight of *S. rubra* plants were recorded at 200 mM NaCl and the growth declined with a further increase in salinity. Kong and Zheng (2014) recorded that the fresh and dry weights of *S. bigelovii* were increased significantly until it reaches the maximum at 200 mM NaCl. Park *et al.* (2013) found that based on the FW, the growth rate of *S. europaea* increased more at 100 mM NaCl

than it did in 0 and 700 mM NaCl. The measurements of stem diameter (table 2) showed that the highest values 2.24mm recorded at 100 and 200 mM NaCl in the root medium. The increase in stem diameter may be achieved by an increase in size of the cells and the relative size of their vacuoles or an increase in the number of cell layers (Shabala and Mackay, 2011). Also consistent with our results, Ayala and O'Leary, (1995), in their study on *S. bigelovii* indicated that plants grown at suboptimal salinity had significantly smaller stem diameters, possibly because of a reduction in cell size.

Table 3. The results of analysis of variation for common glasswort (*Salicornia europaea*).

		Means of squares					
Source of variation	df	Plant high (cm)	Root length (cm)	Lateral branches (no)	Fresh weight (g/plant)	Stem diameter (mm)	Node Length (mm)
Treatment	5	59,422**	318,756**	291,156*	2508,947**	0,001**	13,956**
Error	12	5.67	20.33	63.78	18.73	3,88	0,56
Total	17						

* and ** show significance at the0.05 and 0.01probability level, respectively; and ns shows non-significance.

Table 4. Effect of salinity stress on protein content (%) and proline content (µmoles/g FW) of common glasswort (*Salicornia europaea*).

Treatments	NaCl (mM)	Protein Content (%)			Proline content (µmoles/g FW)		
		M		SD	M		SD
1	0	0.079	±	0.03	0.16	±	0.04
2	100	0.098	±	0.02	0.21	±	0.06
3	200	0.099	±	0.01	0.33	±	0.02
4	300	0.128	±	0.03	0.48	±	0.06
5	400	0.137	±	0.03	1.20	±	0.03
6	500	0.140	±	0.01	1.27	±	0.03

*The data represent mean ± SD of three replicates, **M= Mean, SD= Standard deviation.

The smaller cells may be a consequence of reduced turgor pressure resulting from a low vacuolar content of Na^+ and Cl^-, because Na^+ and Cl^- may be preferentially accumulated in cell walls when the availability of NaCl is limited (Rozema and Schat, 2013). Data in Table 2 showed that the same trend was observed with node length (mm) of *S. europaea,* which was increased with increasing salinity stress until it reaches the maximum at concentrations of 100 and 200 mM NaCl and then gradually decreased until it reaches the very least, with concentration of 500 mM NaCl. Kong and Zheng (2014) showed that the number of nodes and side branches on the main stem of *S. bigelovii* did not significantly differ among the NaCl treatments. It is clear from this study and others that salinity can stimulate growth in halophytes (Flowers and Colmer, 2008) up to a point, followed by declines in growth with further increases in salinity. The mechanisms by which Na^+ first stimulates then represses the growth of halophytes over their tolerance range remain unclear (Tester and Davenport, 2003). The analysis of variance related to the effects of NaCl level on plant high, root length, lateral branches, fresh weight, nod length and stem diameter is given in Table 3. The difference between NaCl concentrations was significant ($p<0.05$) on plant high, root length, fresh weight and stem diameter except for lateral branches. On the other hand, the lateral branch number was significant at the $P<0.01$ probability level.

Effect of salinity stress on physiological parameters

Effect of salinity on protein content

Our results indicated that protein content remained

close to control at moderate NaCl concentrations (100 and 200 mM) and increased at higher salinities. These results are in agreement with Parks *et al.* (2002) who observed that protein accumulation was increased in *S. bigelovii* grown in high concentrations of NaCl. In contrary Aghaleh *et al.* (2009) found that content of proteins reduced in *S. persica* and *S. europaea* species under salt stress.

Table 5. Effect of salinity stress on antioxidant enzymes activity (unites) of common glasswort (*Salicornia europaea*).

Treatments	NaCl (mM)	POD (µ mol min^{-1} mg $^{-1}$ protein)			SOD (U mg^{-1} protein)			CAT (µ mol min^{-1} mg $^{-1}$ protein)		
		M		SD	M		SD	M		SD
1	0	17.24	±	8.2	12.00	±	3.1	4.32	±	0.69
2	100	19.20	±	7.5	13.15	±	3.1	15.89	±	0.29
3	200	34.80	±	16.9	16.05	±	3.1	33.24	±	0.95
4	300	47.45	±	6.6	16.32	±	3.1	12.22	±	0.47
5	400	54.57	±	9.0	23.87	±	1.7	10.19	±	0.56
6	500	60.61	±	2.3	29.21	±	3.1	9.89	±	0.83

*The data represent mean ± SD of three replicates, **M= Mean, SD= Standard deviation.

Effect of salinity on free proline content

Proline is a dominant organic molecule that accumulates in many organisms upon exposure to environmental stress (Aghaleh *et al.*, 2011) and plays multiple roles in plant adaptation to stress (Nanjo *et al.*, 1999). Data presented in Table 4 showed that the proline content in leaves significantly influenced by salinity levels. On the other hand, the free proline content was significantly enhanced in the stressed plants over control plants. Also, there was a considerable increase in free proline accumulation with increasing salt stress. A more pronounced increase was observed under the 500 mM NaCl. These results are in agreement with Aghaleh *et al.* (2011) who found that salinity increased proline content in *S. persica* and *S. europaea* species as compared to control. They also recorded a significant correlation between salinity tolerance and an increase of proline concentration in adjacent branch tissue in the seedlings apexes of *Salicornia* after been exposed to salinity conditions. Many plants accumulated proline as nontoxic and protective osmolyte under saline conditions (Siddiqui *et al.* 2009; Khan *et al.* 2010). Proline is also considered to be involved in the protection of the enzymes (Solomon *et al.*, 1994), cellular structures (Van Resenburg *et al.* 1993), and to act as a free radical scavenger. Park *et al.* (2013) reported that *S. europaea* needs NaCl for normal growth, and osmotic solutes, such as proline accumulate in the plant cell under high NaCl level, to maintain the balance of osmotic potential induced by NaCl accumulated in vacuoles.

Table 6. The results of analysis of variance of common glasswort (*Salicornia europaea*).

		Means of squares				
Source of variation	df	Protein Content (%)	Proline content (µg/g FW)	POD activity (µ mol min^{-1} mg $^{-1}$ protein)	SOD activity (U mg^{-1} protein)	CAT activity (µ mol min^{-1} mg $^{-1}$ protein)
Treatment	5	1264,402*	7520,325^{ns}	998,197**	135,123*	6522,824**
Error	12	37,60	67,82	89,75	32,46	47,20
Total	17					

* and ** show significance at the 0.05 and 0.01probability level, respectively; and ^{ns} shows non-significance.

Effect of salinity on antioxidant enzyme activity

The activities of peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) in *S. europaea* under the effect of different NaCl concentrations are given in table 5. In general, salt treatments increased antioxidant enzyme activities in *Salicornia* plants. POD, SOD, and CAT activates were increased gradually with increasing salinity stress.

POD

In the present study, with the increase of NaCl content to solution, POD activity increased by 100, 200, 300, 400 and 500 mM NaCl in comparison with the control. There was a significant difference (P<5%) between the control and other treatments. POD activity at 200 mM increased severely in comparison with control. However, salt stress at the highest NaCl levels slightly increased POD activity. The maximum POD activity was obtained at 500 mM NaCl, which was 252% of the control. These results contrast to the results of Cao *et al.* (2015), who found that POD activity decreased significantly in *Suaeda aralocaspica* under higher salinity.

SOD

In our study, the activities of SOD in *S. europaea* gradually increased with increasing NaCl concentrations, and the highest SOD activity was determined at 500 mM NaCl, which was 143% of the control values. These results are in strong confirmation with the results of Aghaleh *et al.* (2011) who reported quantitatively higher SOD activities in two species of *Salicornia* under the effect of different NaCl concentrations. They also found that, the total SOD activities in both species gradually increased with increasing NaCl concentrations. Demiral and Turkan (2004) reported quantitatively higher SOD activities in salt-tolerant Pokkali than salt sensitive IR-28. Cao *et al.* (2015), the activity of SOD significantly increased under higher salinity treatments (500 mM Nacl).

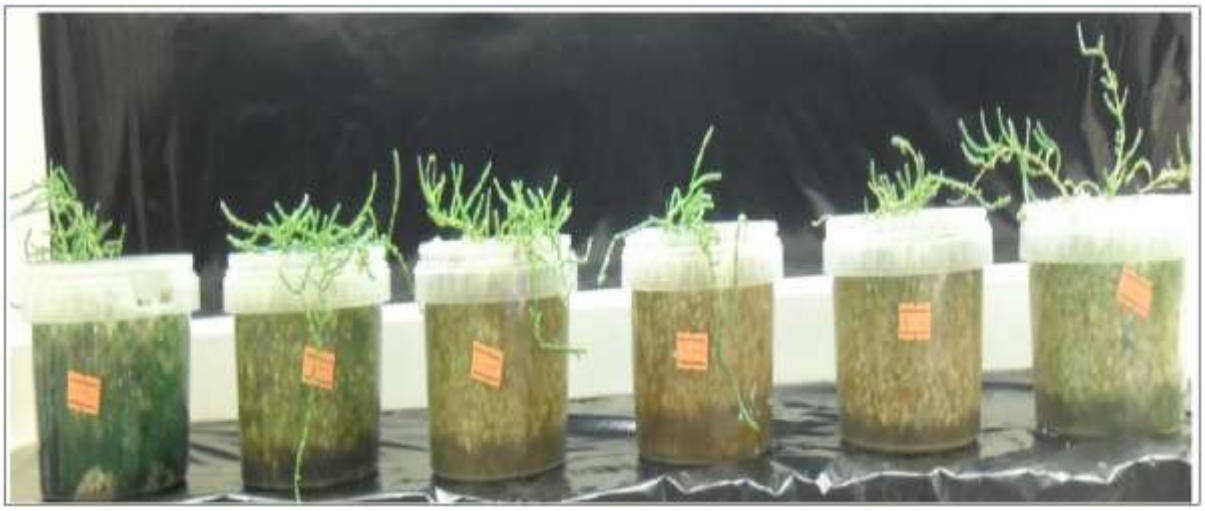

Fig. 1. Effect of various NaCl concentrations on growth of *Salicornia europaea* after 60 days of treatment: seedlings of *Salicornia* subjected to 0, 100, 200, 300, 400 and 500 mM NaCl.

CAT

It is another important antioxidant enzyme that converts H_2O_2 to water in the peroxisomes (Fridivich, 1989). In this organelle, H_2O_2 is produced from β-oxidation of fatty acids and photorespiration (Morita *et al.*, 1994). Higher activity of CAT decrease H_2O_2 level in cell and increase the stability of membranes and CO_2 fixation because several enzymes of the Calvin cycle within chloroplasts are extremely sensitive to H_2O_2. A high level of H_2O_2 directly inhibits CO_2 fixation (Yamazaki *et al.*, 2003). The catalase activity was significantly increased in *S. europaea* at 100 and 200 mM NaCl in comparison with the control. But at higher levels of NaCl, i.e., 300, 400 and 500mM, catalase activity significantly decreased (P<5%) in comparison with control plants. The results of catalase are in agreement with those obtained by Cao *et al.* (2015), who reported that the activity of SOD significantly increased with the rising O^{2-}, while that of CAT decreased significantly in *Suaeda aralocaspica* plants under higher salinity, suggesting that the failure of an increase in the activity of CAT might mean that the excess H_2O_2 was not effectively scavenged, thereby causing more serious oxidative stress. Aghaleh *et al.* (2011) recorded that POD, and SOD activities play an

essential protective role in the scavenging reactive oxygen species (ROS) in *S. persica* and *S. europaea* species. The analysis of variance (ANOVA) for protein content, proline content, POD, SOD and CAT activities related to the effects of NaCI is given in Table 6. The difference between NaCl concentrations was significant ($p<0.05$) on POD and CAT activity. However, the effect of NaCl addition was not significant on proline content. On the other hand, protein content and SOD activity were significant only at $p<0.01$ probability level.

Conclusion

Based on the data obtained from plant growth and physiological parameters, it is clear that the halophytic characteristics of *Salicornia europaea* plants as evidenced by the positive effect of moderate salinities on plant growth. There was a gradual increase in most parameters with increasing the concentrations of salts until it reached the maximum at a concentration of 200 mM NaCl. Then a gradual decline happened until it reached the lowest at a concentration of 500 mM NaCl. POD and SOD activities play an essential protective role in the scavenging reactive oxygen species (ROS) in *S. europaea*.

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