Acta Bot. Croat. 81 (2), 213–220, 2022
DOI: 10.37427/botcro-2022-019

CODEN: ABCRA 25
ISSN 0365-0588
eISSN 1847-8476

Exogenous arginine treatment additively enhances growth and tolerance of *Salicornia europaea* seedlings under salinity

Fereshteh Shakhsi-Dastgahian[1], Jafar Valizadeh[1], Monireh Cheniany[2], Alireza Einali[1]*

[1] University of Sistan and Baluchestan, Faculty of Science, Department of Biology, Zahedan, Iran

[2] Ferdowsi University of Mashhad, Faculty of Science, Department of Biology, Mashhad, Iran

Abstract – The effects of foliar application of 5 mM arginine (Arg) on the growth and control of salinity-induced osmotic and oxidative stresses (0, 200, 400 and 600 mM NaCl) in *Salicornia europaea* seedlings were investigated. Despite higher levels of lipid peroxidation, lower membrane stability index (MSI), decreased pigment content and phenolic compounds, and reduced activity of antioxidant enzymes observed under salinity, seedling growth indices, including plant height and biomass, increased significantly, and some protective and antioxidant molecules such as proline and flavonoids accumulated. Soluble protein level increased at the low salt concentration (200 mM) but decreased at other doses. Exogenous Arg treatment alone had less or no effect on plant biomass and other metabolites, but in combination with salt, further enhanced growth parameters, MSI and accumulation of soluble protein, phenolic compounds and proline. Arg-induced changes under salinity were associated with decreased lipid peroxidation, flavonoids content and antioxidant enzymes activity. These results show that *S. europaea* seedlings are well tolerant to applied salt doses. The treatment with exogenous Arg alone affects plant growth slightly, but in combination with salt, synergistically increases growth and salt tolerance of these plants by enhancing the accumulation of proline and antioxidant molecules instead of enzymatic antioxidant.

Keywords: arginine, lipid peroxidation, *Salicornia europea*, salinity, proline

Introduction

In arid and semi-arid regions, lack of rainfall and high temperatures have increased soil salinity. Because most plants are sensitive to high salt contents, their growth and yield are affected by the osmotic and oxidative stresses induced by saline soils (Li et al. 2011, Wu et al. 2012). Production of reactive oxygen species (ROS) due to oxidative stress is an inevitable process in plants exposed to salt and other environmental stresses (Allen 1995, Garg and Manchanda 2009). Increased ROS levels under salinity stress damage plant metabolism and destroy cell membrane lipids and proteins as well as other biomacromolecules (Bor et al. 2003, Gill and Tuteja, 2010), which subsequently alters selective membrane permeability and causes the material to leak out of the cell (Weckx and Clijsters 1997). In saline areas, one of the best ways to reduce the harmful effects of salinity and increase productivity is to cultivate salt-resistant plants (Oba et al. 2001). Many halophyte plants can grow in saline soils due to changes in their energy metabolism (Winicov and Bastola 1997). The salinity tolerance mechanisms of halophytes are not well understood but may be due in part to the synthesis of proline and other osmolytes, ion homeostasis, and the activity of the antioxidant defense system to scavenge ROS (Hasegawa et al. 2000).

L-arginine (Arg) is one of the proteinogenic amino acids of plant cells, which is a precursor to the synthesis of proline, polyamines, and nitric oxide (Liu et al. 2006). Arg treatment improved chlorophyll synthesis and photosynthesis and prevented chlorophyll degradation and ageing (Zeid 2009, Mostafa et al. 2010). Exogenously applying Arg in plants exposed to environmental stresses upregulated antioxidant enzymes (Barand et al. 2015) and induced the accumulation of compatible solutes (Ramadan et al. 2019).

Salicornia europea L. is a halophyte plant that is widely distributed in coastal areas and salt marshes (Davy et al. 2001). Because it can survive in salt concentrations that are toxic to most plants, *Salicornia* is known as a salt-tolerant plant species (Flowers and Colmer 2008, Patel 2016). It is taken not only as a model species for salinity research, but

* Corresponding author e-mail: aeinali@science.usb.ac.ir

it has also recently been considered as a potential food and pharmaceutical plant due to the accumulation of suitable nutrients such as polyphenols, fibres, and flavonoids (Patel 2016). Despite some studies on salt stress and the role of Arg in its tolerance (Nejadalimoradi et al. 2014, Ramadan et al. 2019), there is no report on the possible effects of this amino acid on salt stress tolerance in *Salicornia*. Therefore, the present work aimed to evaluate the impact of exogenous Arg on the growth and control of osmotic and oxidative stresses caused by salinity in *S. europea*.

Materials and methods

Plant materials and experimental design

Uniform seeds of *Salicornia europaea* L. were obtained from the Pakan Bazr Company at Esfahan. Seeds soaked in tap water for 12 h were planted in trays filled with wet cocopeat and placed at 28 ± 1 °C to germinate. The 4 to 5 cm seedlings were transferred to plastic pots (two seedlings per pot) containing 1 kg of well-watered cocopeat-perlite (2:1) in a phytotron at 25 °C, a relative humidity of 60%, and a photoperiod of 16 h light/ 8 h dark. The seedlings were fed with 1/2 Hoagland's nutrient solution at three-day intervals until their length reached 20 to 23 cm. Three days before salinity treatment, the seedlings were divided into two groups of 20 pots containing 40 seedlings. One group was treated with foliar application of 5 mM Arg, and the other group was left untreated and just received distilled water. Both groups were exposed to four levels of NaCl (0, 200, 400, and 600 mM) by the addition of salt to the 1/2 Hoagland's nutrient solution. Salt and Arg treatments were applied to seedlings at three-day and six-day intervals for up to 30 days, respectively. Seedlings were then harvested and evaluated for morphological and biochemical responses to salinity and Arg treatments. All studies were independently repeated using separate seedlings at least three times for each morphological and biochemical analysis.

Assessment of morphological traits and membrane stability index (MSI)

The measured weight characteristics included fresh weight (FW) and dry weight (DW) of both shoot and root, and longitudinal traits included shoot and root length.

MSI was determined according to Sairam and Saxena (2000). Two samples of fresh shoot tissue (0.1 g) were placed separately in a test tube containing 10 ml of distilled water. One sample was heated in a water bath at 40 °C for 30 min, and the other sample was boiled at 100 °C for 10 min. After determination of the electrical conductivity (EC) of the samples, MSI was calculated by the following formula:

$$MSI=1-(EC_{40}/EC_{100}) \times 100$$

Determination of photosynthetic pigments

Chlorophyll (Chl) and carotenoids (Car) were extracted from 0.1 g of fresh shoot tissue with 2 ml of 95% (v/v) ethanol. The extracts were filtered through a Whatman No.1 filter paper, and their absorbance was read at 664, 648, and 470 nm to measure Chl *a*, Chl *b*, and Car, respectively (Lichtenthaler and Buschmann 2001). Total Chl was obtained from the sum of Chl *a* and Chl *b*. Pigment contents were expressed in mg per g fresh weight.

Enzyme extraction and assay

Fresh shoot tissue (0.1 g) was ground in liquid nitrogen and homogenized with 1 ml of enzyme extraction buffer containing 100 mM cold potassium phosphate buffer (pH 7.4), 1 mM EDTA, 5 mM ascorbate, 50 mM 2-mercaptoethanol, and 1% (w/v) polyvinylpolypyrrolidone (PVPP). The homogenate was filtered through three layers of cheesecloth and centrifuged at 12,000 *g* for 10 min at 4 °C. Decoloration of the extract was done by adding 10 mg of charcoal before centrifugation. The 12,000 *g* supernatant was used for enzyme assay and soluble protein determination. Soluble protein content was determined by Bradford's method (1976) using bovine serum albumin (BSA) as a standard.

Ascorbate peroxidase (APX) activity was determined spectrophotometrically by measuring the generation rate of dehydroascorbate in a reaction mixture (1 ml) containing 50 mM potassium phosphate buffer (pH 7.0), 1 mM H_2O_2, 0.5 mM ascorbic acid, and enzyme extract at 290 nm, assuming an extinction coefficient of 2.8 mM^{-1} cm^{-1} (Chen and Asada 1992).

Peroxidase (POX) activity was determined by evaluating the rate of guaiacol oxidation in an assay mixture (1 ml) containing 50 mM potassium phosphate buffer (pH 7.0), 5 mM guaiacol, 1 mM H_2O_2, and enzyme extract at 470 nm, using an extinction coefficient of 26.6 mM^{-1} cm^{-1} (Srinivas et al. 1999).

The assay of polyphenol oxidase (PPO) was done according to the rate of purpurogallin production in 1 ml of a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 40 mM pyrogallol, and enzyme extract. The assay mixture was monitored at 430 nm, and the activity of the enzyme was calculated using the extinction coefficient of 2.47 mM^{-1} cm^{-1} (Nakano and Asada 1981).

Determination of total phenolic and flavonoid contents

Phenol compounds were determined by the method of Singleton et al. (1999) with some modifications (Einali et al. 2018) using gallic acid as the standard.

Total flavonoids were measured according to Krizek et al. (1998). Fresh shoot tissue (0.1 g) was extracted with 1 ml of acidified ethanol (ethanol: acetic acid, 99:1, v/v). The extract was centrifuged at 5,000 *g* for 10 min, and the absorbance of the resultant supernatant was recorded at 300 nm after heating at 80 °C for 10 min. Total flavonoid content was expressed as absorbance per mg of shoot tissue FW.

Other analytical methods

Proline content was estimated according to Bates et al. (1973) using proline as a standard. The level of lipid peroxidation was determined by measuring malondialdehyde (MDA) content as described by Heath and Packer (1968), assuming an extinction coefficient of 155 $Mm^{-1}cm^{-1}$.

Tab. 1. Exogenous effect of 5 mM arginine (Arg) on plant longitudinal characteristics, plant biomass and membrane stability index (MSI) of *Salicornia europaea* seedlings under different concentrations of salinity. Each value is the mean ± SE of three independent measurement from three separate seedlings and the different letters in each column indicate significant differences among the various treatments at $P < 0.05$ according to the Duncan test.

Salt treatment (mM)	Arginine treatment	Shoot length (cm)	Root length (cm)	Fresh weight (g)		Dry weight (g)		MSI (%)
				Shoot	Root	Shoot	Root	
0	-Arg	13.70±0.87c	9.97±0.61d	1.23±0.09f	0.23±0.03c	0.01±0.002g	0.018±0.001e	48.30±4.10a
	+Arg	16.90±2.24c	13.00±0.93c	2.76±0.28c	0.25±0.07c	0.04±0.003c	0.029±0.002d	51.10±6.30a
200	-Arg	16.00±2.00c	11.97±1.05c	1.88±0.33e	0.57±0.06b	0.02±0.003f	0.021±0.004e	43.70±1.30b
	+Arg	23.30±1.31b	15.60±0.80b	3.63±0.23b	0.29±0.06c	0.04±0.002bc	0.035±0.003d	46.80±2.60ab
400	-Arg	21.57±2.79b	14.43±1.62b	2.22±0.16e	0.59±0.10b	0.03±0.003d	0.032±0.005d	42.10±3.30b
	+Arg	27.43±1.67a	17.77±0.38a	4.80±0.24a	0.26±0.03c	0.05±0.003a	0.060±0.002a	45.20±1.20b
600	-Arg	19.80±1.91b	14.03±1.04b	2.68±0.19d	0.96±0.10a	0.03±0.003e	0.042±0.003c	18.80±2.10c
	+Arg	22.70±2.35b	15.77±0.94b	3.39±0.25b	0.22±0.08c	0.04±0.004ab	0.053±0.004b	44.70±4.40b

Statistical analysis

All data were expressed as the mean ± standard error (SE) of triple analyses. Normality and equal variance were also tested. Statistically significant differences were determined in a factorial design by a two-way analysis of variance (ANOVA) at P < 0.05 using the Duncan method.

Results

Effect of exogenous Arg on plant growth and membrane stability during salt treatment

Salt and Arg treatments alone or in combination increased the apparent growth of *S. europaea* seedlings (Tab. 1, On-line Suppl. Fig. 1). Both shoot and root lengths were

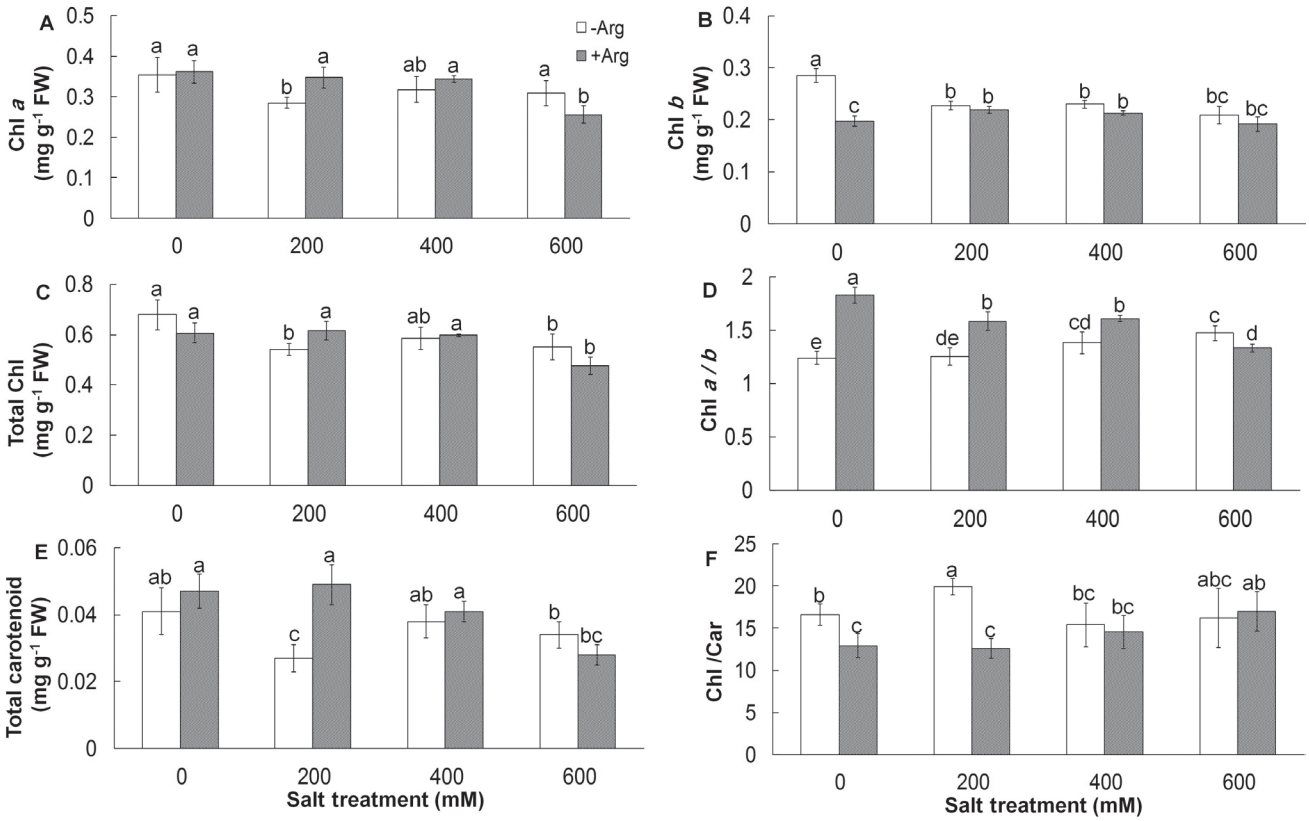

Fig. 1. Changes in the content of the photosynthetic pigments of *Salicornia europaea* seedlings under salinity treated with or without 5 mM Arg. (A) Chl *a*, (B) Chl *b*, (C) Total Chl, (D) Chl *a*/*b*, (E) Total Car, and (F) Chl/Car ratio. Data are mean ± SE of three independent experiments from three separate seedlings, and the different letters indicate significant differences among the various treatments at $P < 0.05$ according to the Duncan test.

positively affected by salinity in a concentration-dependent manner. The highest increase in length was found in seedlings treated with a concentration of 400 mM NaCl. Arg treatment showed a significant effect on improving plant height either alone or in combination with salinity. However, shoot and root height remained statistically unchanged in Arg-treated seedlings grown at 600 mM NaCl compared to untreated controls (Tab. 1).

The fresh and dry weight of seedlings increased in response to salt treatment in shoot and root. Plant biomass and shoot fresh weight were positively changed by Arg treatment alone or in combination with salinity. However, fresh weight of root in Arg-treated seedlings remained unchanged or was drastically decreased compared to untreated controls (Tab. 1).

Salt treatment alone reduced MSI at all concentrations, especially in seedlings treated with 600 mM NaCl. Arg treatment alone or in combination with salinity up to 400 mM did not affect MSI but greatly improved this index in seedlings treated with 600 mM NaCl (Tab. 1).

Effect of exogenous Arg on plant pigments during salt treatment

Salt treatment at all concentrations significantly reduced the photosynthetic pigments of *S. europaea* seedlings (Fig. 1). Arg treatment alone did not change Chl *a* content, but its effect in combination with salinity depended on the salt concentration (Fig. 1A). In contrast, Arg treatment alone significantly reduced Chl *b* levels but had no effect when applied in combination with salinity (Fig. 1B). Total Chl content was positively affected by Arg treatment only in seedlings grown at 200 mM NaCl (Fig. 1C). The Chl *a*/*b* ratio increased in response to both salinity and Arg treatments (Fig. 1D). However, this ratio decreased in Arg-treated seedlings grown in 600 mM NaCl.

Arg treatment enhanced carotenoid content in 200 mM NaCl-grown seedlings but did not change it when applied alone or in combination with other salinities (Fig. 1E). The Chl / Car ratio was affected by 200 mM NaCl but not by other salt concentrations. Arg treatment alone or in combination with 200 mM NaCl reduced this ratio but was not effective at other salt doses (Fig. 1F).

Effect of exogenous Arg on plant phenolics during salt treatment

Salt treatment at all concentrations caused a significant reduction in the total phenol concentration of *S. europaea* seedlings (Fig. 2A). Arg treatment alone decreased phenol content, while it was effective in a salt-dependent manner when applied to seedlings grown at different salinity concentrations. In contrast, the flavonoid content of seedlings grown at 400 and 600 mM NaCl enhanced compared to the control (Fig. 2B). While Arg treatment reduced the level of seedling flavonoids exposed to the mentioned salt doses, it was ineffective when used alone or with 200 mM NaCl (Fig. 2B).

Effect of exogenous Arg on proline, soluble protein and lipid peroxidation during salt treatment

The proline content in seedlings grown at salinity up to 400 mM remained statistically unchanged but increased significantly in response to 600 mM NaCl (Fig. 3A). Arg

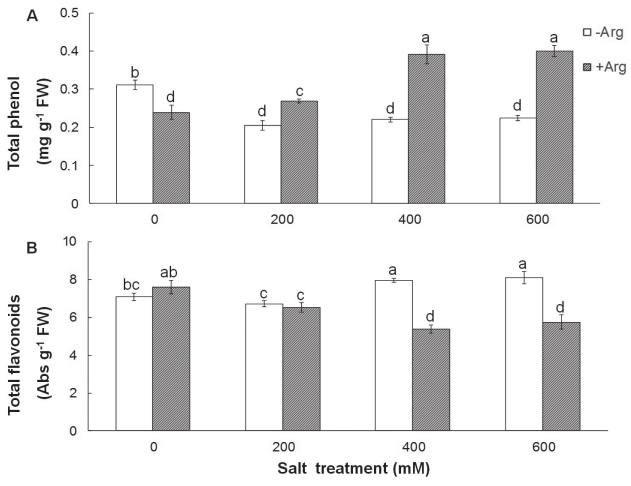

Fig. 2. Changes in total phenol (A) and total flavonoid (B) contents of *Salicornia europaea* seedlings under salinity treated with or without 5 mM arginine (Arg). Values are mean ± SE of three independent experiments from three separate seedlings. The different letters show significant differences among the various treatments at $P < 0.05$ according to the Duncan test.

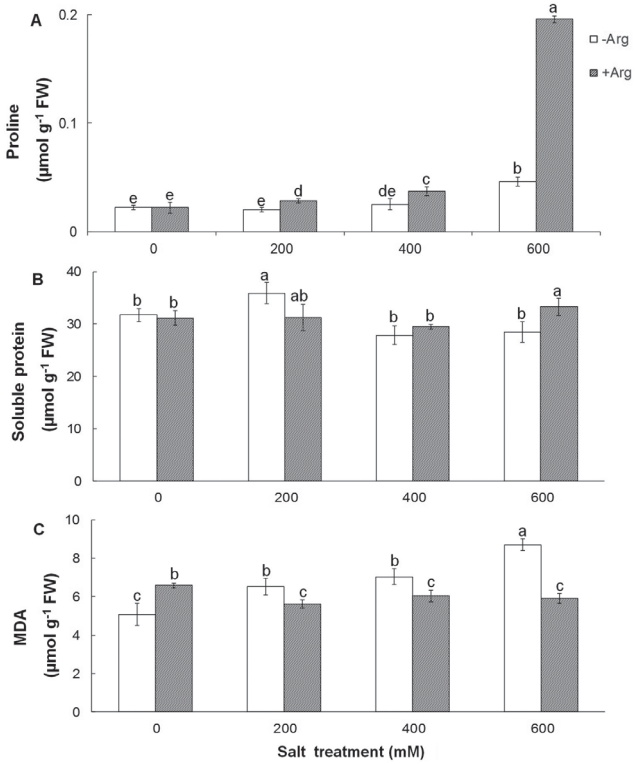

Fig. 3. Changes in proline (A), soluble protein (B), and malondialdehyde (MDA) (C) contents of *Salicornia europaea* seedlings under salinity treated with or without 5 mM arginine (Arg). Data are mean ± SE of three independent experiments from three separate seedlings. The different letters indicate significant differences among the various treatments at $P < 0.05$ according to the Duncan test.

treatment alone did not change proline levels, whereas this metabolite was highly accumulated when combined with salt. Arg-treated seedlings grown in 600 mM NaCl showed more than 4-fold proline accumulation than untreated controls (Fig. 3A).

Soluble protein concentration increased in 200 mm NaCl-grown seedlings but decreased in seedlings fed higher doses of salt. Protein content was not affected by Arg treatment alone or with salt up to 400 mM, whereas when combined with 600 mM NaCl, it changed positively (Fig. 3B).

Lipid peroxidation was increased by salinity in a dose-dependent manner. Arg treatment increased the MDA content in seedlings nourished with salt-free medium but decreased it in plants grown in combination with salt (Fig. 3C).

Changes in enzyme activities in Arg-treated seedlings during salt treatment

The activities of APX, POX and PPO in *S. europaea* seedlings were negatively changed by salt treatments (Fig. 4). Exogenous Arg treatment highly decreased the APX activity of salt-treated or salt-untreated seedlings (Fig. 4A). However, POX activity of seedlings treated with Arg alone or in combination with salinity up to 400 mM remained roughly unchanged but decreased abruptly in seedlings growing at 600 mM NaCl compared to untreated controls (Fig. 4B). In contrast, PPO activity was positively affected by Arg treatment alone or in combination with salt (Fig. 4C).

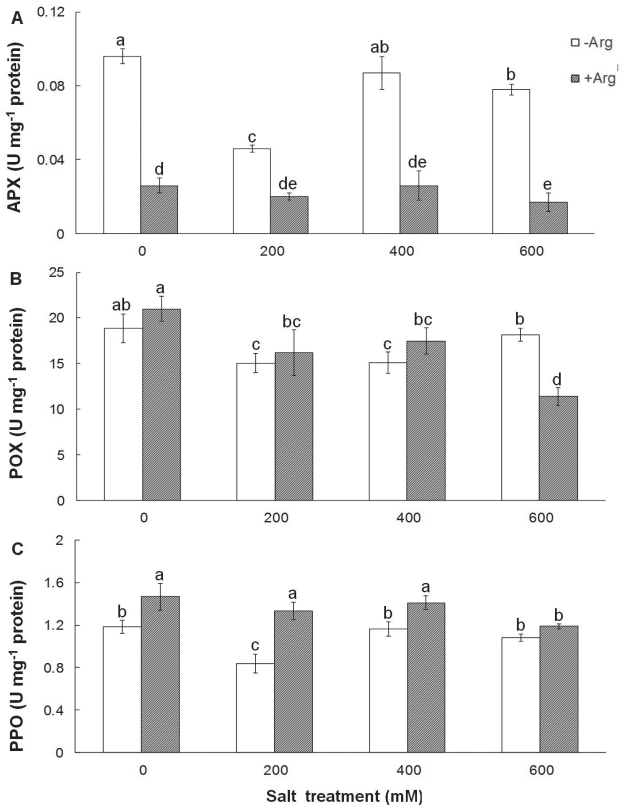

Fig. 4. Changes in the activity of ascorbate peroxidase (APX) (A), peroxidase (POX) (B), and polyphenol oxidase (PPO) (C) of *Salicornia europaea* seedlings under salinity treated with or without 5 mM arginine (Arg). Results are mean ± SE of three independent experiments from three separate seedlings. The different letters show significant differences among the various treatments at $P < 0.05$ according to the Duncan test.

Discussion

In contrast to the destructive effects of salinity on plant growth and biomass in most plants (Qiu et al. 2014, Ahmad et al. 2018, Ghanem et al. 2021), our results showed that salinity could have a positive effect on height, fresh weight, and biomass of *S. europaea* seedlings. Similarly, the maximum growth of another *Salicornia* species (*S. dolichostachya*) was observed at 300 mM NaCl (Katschnig et al. 2013), which is consistent with our results. Arg treatment alone or with salt has an additive effect on plant growth and biomass. This agrees with most studies showing that foliar spraying of mung bean (Qados 2010), wheat (Mostafa et al. 2010), and sunflower (Nejadalimoradi et al. 2014, Ramadan et al. 2019) with Arg increased the growth of these plants under salinity stress. The ameliorative effect of Arg on plant growth under salinity has been attributed to its role as a precursor of polyamines (Mostafa et al. 2010) or as a source of nitric oxide (NO) (Ramadan et al. 2019). However, the negative effect of Arg on root FW during salinity was noticeable. In contrast to the synergic effects of Arg and salinity on plant growth, root FW showed an antagonistic relationship between Arg and salinity treatments. Due to the enhancement effect of Arg and salt treatments on root biomass, this diminishing effect may refer to the potential for water maintenance in root tissues of salt-treated seedlings in the presence of Arg.

Reduction of photosynthetic pigments of *S. europaea* occurred under salt treatment, which is consistent with other studies (Zeid 2009, Ramadan et al. 2019, Ghanem et al. 2021). The decrease in pigment content under salinity can be attributed to their degradation by free radicals generated during stress (Ma et al. 2020) or activation of chlorophyll catabolic processes along with inhibition of biosynthetic enzymes (Rady et al. 2015). Unlike other studies (Zeid 2009, Ramadan et al. 2019), Arg treatment alone did not affect or decrease the pigment content of *S. europaea* seedlings. The positive effect of Arg on pigment content was observed only with 200 mM NaCl. This implies that Arg does not change photosynthetic pigments under high salinities. Thus, the improvement in the growth of Arg-treated seedlings under salinity is not directly related to photosynthetic pigments but may be due to the increased photosynthetic capacity through maintaining chloroplast structure. The reduction of lipid peroxidation during salinity due to Arg treatment associated with an increase in MSI could further support this suggestion.

One of the effects of salinity is lipid peroxidation, which occurs in plants both sensitive to and tolerant of salinity, due to oxidative damage, although its severity is higher in salt-sensitive plants (Kumar et al. 2020). Accumulation of MDA, as an indicator of lipid peroxidation, in *S. europaea* seedlings under salinity was associated with a decrease in MSI, which indicates the destructive effect of salt. However,

these seedlings had a higher growth in the presence of salt, showing that they can tolerate these salinity concentrations well. Arg treatment in combination with salt but not alone, caused less lipid peroxidation and increased MSI. Similar results were reported in canola seedlings treated with Arg under salinity (Nasibi et al. 2014). This indicates that Arg enhances salt tolerance in *S. europaea*. To counteract the oxidative damage caused by abiotic stress, plants use defense mechanisms, including the accumulation of Osmo protectants and non-enzymatic compounds along with antioxidant enzymes (Ali et al. 2017, Polash et al. 2019). Proline is a compatible solute that acts as an Osmo protectant, stabilizer and protector of membranes, enzymes, and proteins, and scavenger of free radical (Ashraf and Foolad 2007, Kumar et al. 2020). As recently demonstrated (Ghanem et al. 2021), the increase in proline during salt treatments of *S. europaea* seedlings indicates the strategy of these plants in salt tolerance. Accumulation of proline in seedlings treated with Arg under salinity can be related to the positive effects of this amino acid on the production of proline, NO and polyamines (Liu et al. 2006). Thus, a more than fourfold increase in the proline content of Arg-treated seedlings exposed to 600 mM NaCl could explain a decrease in lipid peroxidation versus an increase in MSI in these plants.

Polyphenols and flavonoids, as antioxidant molecules, play an important role in scavenging free radicals by themselves and inducing antioxidant enzymes (Kofroňová et al. 2020). Our results showed that the content of total phenols decreases, but the flavonoids increase during salinity. However, increases in both polyphenols and flavonoids have been reported in plants under salt stress (Lim et al. 2012, Sarker et al. 2019). The phenolics changes during salinity were associated with a decrease in PPO activity, which can be related to salt tolerance. This is consistent with studies showing that decreased PPO activity is associated with stress tolerance (Thipyapong et al. 2004, Sofo et al. 2005). It indicates that among phenolic compounds, flavonoids may have a role in *S. europaea* tolerance to salinity. In contrast, Arg treatment combined with salinity had an additive effect on total phenols but decreased flavonoids content along with an increase in PPO activity. Considering the role of Arg in enhancing the growth of *S. europaea* seedlings under salinity, Arg treatment probably induces the production of phenolic compounds other than flavonoids to scavenge free radicals. It means that Arg with salt can change the pattern of phenolic production with PPO activity to induce high salt tolerance. This result in conflict with PPO activity points to the unclear role for the enzyme in stress tolerance, as reported previously (Boeckx et al. 2015).

The soluble protein content of *S. europaea* increased at 200 mM NaCl but decreased under more salt concentrations (400 and 600 mM). This is consistent with previous reports of *Pisum sativum* (Velitcukova and Fedina 1998) and tomato (Manan et al. 2016) under salt stress, indicating a decrease in soluble protein levels due to inhibition of protein biosynthesis or increased protein degradation (Mersie and Singh 1993). Arg treatment increased the protein levels of seedlings fed with 600 mM NaCl. The positive effect of Arg on the increase of soluble proteins, which has also been reported in *Lupinus termis* under salinity (Akladious and Hanafy 2018), refers to the synthesis of specific proteins that are involved in the salinity tolerance of plants (Qados, 2010).

In contrast to the findings of most studies (Ali et al. 2017, Polash et al. 2019, Kumar et al. 2020), in this research the activity of antioxidant enzymes, such as APX and POX as H_2O_2-decomposing enzymes in *S. europaea* seedlings decreased or remained unchanged in response to different salt concentrations. Similarly, Arg treatment decreased APX activity at all salinities but reduced POX at only 600 mM NaCl. Since Arg is involved in the production of proline, NO and polyamines (Liu et al. 2006), its protective effect, increasing salt tolerance in *S. europaea* seedlings, can be attributed to the subsequent metabolism of this amino acid. There are two lines of evidence that support this suggestion. First, the positive effect of Arg on proline accumulation, especially in 600 mM NaCl, confirms the role of Arg in inducing proline biosynthesis. The second line of evidence comes from a study showing that the protective effect of Arg on sunflower seedlings under salt stress may be due to the release of NO from Arg (Nejadalimoradi et al. 2014). However, in contrast to this report, our results show that Arg treatment decreased the activity of APX and POX in seedlings under salinity. Therefore, the role of Arg in enhancing the salt tolerance of this plant may be related to proline accumulation.

In the current study, salinity *per se* played a positive role in the growth and biomass of root and shoot in *S. europaea* seedlings, demonstrating that they tolerate these salt doses. Salt treatment was also associated with increased lipid peroxidation and decreased MSI, pigment content and phenolic compounds. Soluble protein content increased at low salinity but decreased under other salt concentrations. While the activity of antioxidant enzymes decreases, proline and flavonoids accumulate during salinity. Arg treatment alone exhibited a slight or no change in seedling growth and metabolite content, but in combination with salt showed a synergistic effect on enhancing growth parameters, MSI and accumulation of soluble protein, phenolic compounds and proline. It was associated with decreased lipid peroxidation, flavonoids and enzyme activity. It can be concluded that Arg treatment alone slightly impresses seedlings growth but when combined with salt, evidently plays an additive role in increasing salt tolerance of these plants by enhancing the accumulation of proline as an osmoprotectant and antioxidant molecules rather than enzymatic antioxidant.

Acknowledgements

We would like to thank the Deputy of Research at the University of Sistan and Baluchestan for financial support in the form of a grant for an M.Sc. research project.

References

Ahmad, P., Alyemeni, M.N., Ahanger, M.A., Wijaya, L., Alam, P., Kumar, A., Ashraf, M., 2018: Up-regulation of antioxidant and glyoxalase systems mitigates NaCl stress in *Brassica juncea* by supplementation of zinc and calcium. Journal of Plant Interactions 13, 151-162.

Akladious, S.A., Hanafy, R.S., 2018: Alleviation of oxidative effects of salt stress in white lupine (*Lupinus termis* L.) plants by foliar treatment with L-arginine. Journal of Animal and Plant Sciences 28, 165-176.

Ali, Q., Daud, M.K., Haider, M.Z., Ali, S., Rizwan, M., Aslam, N., Noman, A., Iqbal, N., Shahzad, M., Deeba, F., Ali, I., Jin, Z.S., 2017: Seed priming by sodium nitroprusside improves salt tolerance in wheat (*Triticum aestivum* L.) by enhancing physiological and biochemical parameters. Plant Physiology and Biochemistry 119, 50–58.

Allen, R.D., 1995: Dissection of oxidative stress tolerance using transgenic plants. Plant Physiology 107, 1049-1054.

Ashraf, M., Foolad, M.R., 2007: Roles of glycine betaine and proline in improving plant abiotic stress tolerance. Environmental and Experimental Botany 59, 206–216.

Barand, A., Nasibi, F., Manouchehri Kalantari, Kh., 2015: The effect of arginine pretreatment in the increase of cold tolerance in *Pistacia vera* L. in vitro. Russian Agricultural Sciences 41, 340–346.

Bates, L.S., Waldren, R.P., Teare, I.D., 1973: Rapid determination of free Proline for water stress studies. Plant and Soil 39, 205-208.

Boeckx, T., Winters, A.L., Webb, K.J., Kingston-Smith, A.H., 2015: Polyphenol oxidase in leaves: is there any significance to the chloroplastic localization? Journal of Experimental Botany 66, 3571–3579.

Bor, M., Ozdemir, F., Turkan, I., 2003: The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritima* L. Plant Science 164, 77-84.

Bradford, M.M. 1976: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248–254.

Chen, G., Asada, K., 1992: Inactivation of ascorbate peroxidase by thoils requires hydrogen peroxide. Plant and Cell Physiology 33, 117–123.

Davy, A.J., Bishop, G.F., Costa, C.S.B., 2001: *Salicornia* L. (*Salicornia pusilla* J. Woods, *S. ramosissima* J. Woods, *S. europaea* L., *S. obscura* P.W. Ball & Tutin, *S. nitens* P.W. Ball & Tutin, *S. fragilis* P.W. Ball & Tutin and *S. dolichostachya* Moss). Journal of Ecology 89, 681–707.

Einali, A., Azizian-Shermeh, O., Ghasemi, A., 2018: Phytochemical screening and antimicrobial activities of *Periploca aphylla* Decne, Persian walnut (*Juglans regia* L.) and oleander (*Nerium indicum* Mill.) Leaf extracts. Journal of Food Measurement and Characterization 12, 1350–1359.

Flowers, T.J., Colmer, T.D., 2008: Salinity tolerance in halophytes. New Phytologist 179, 945–963.

Garg, N., Manchanda, G., 2009: ROS generation in plants: boon or bane? Plant Biosystems 143, 81–96.

Ghanem, A.E.F.M., Mohamed, E., Kasem, A.M.M.A., El-Ghamery, A.A. 2021: Differential salt tolerance strategies in three halophytes from the same ecological habitat: augmentation of antioxidant enzymes and compounds. Plants 10, 1100.

Gill, S.S., Tuteja, N., 2010: Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry 48, 909–930.

Hasegawa, P.M., Bressan, R.A., Zhu, J.K., Bohnert, H.J. 2000: Plant cellular and molecular responses to high salinity. Annual Review of Plant Physiology and Plant Molecular Biology 51, 463-499.

Heath, R.L., Packer, L., 1968: Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics 125, 189–198.

Katschnig, D., Broekman, R., Rozema, J., 2013: Salt tolerance in the halophyte *Salicornia dolichostachya* Moss: growth, morphology and physiology. Environmental and Experimental Botany 92, 32–42.

Kofroňová, M., Hrdinová, A., Mašková, P., Tremlová, J., Soudek, P., Petrová, Š., Pinkas, D., Lipavská, H. 2020: Multi-component antioxidative system and robust carbohydrate status, the essence of plant arsenic tolerance. Antioxidants 9, 283.

Krizek, D.T., Britz, S.J., Mirecki, R.M., 1998: Inhibitory effect of ambient levels of solar UV-A and UV-B radiation on growth of cv. NEW RED FIRE lettuce. Physiologia Plantarum 103, 1–7.

Kumar, S., Li, G., Yang, J., Huang, X., Ji, Q., Zhou, K., Khan, S., Ke, W., Hou, H., 2020: Investigation of an antioxidative system for salinity tolerance in *Oenanthe javanica*. Antioxidants 9, 940.

Li, J.T., Qiu, Z.B., Zhang, X.W., Wang, L.S., 2011: Exogenous hydrogen peroxide can enhance tolerance of wheat seedlings to salt stress. Acta Physiologiae Plantarum 33, 835–842.

Lichtenthaler, H.K., Buschmann, C., 2001: Chlorophylls and carotenoids: measurement and characterization by UV-VIS. In: Wrolstad. R.E. (ed.), Current protocols in food analytical chemistry, F.4.3.1–F.4.3.8. John Wiley and Sons, New York.

Lim, J-H., Park, K-J., Kim, B-K., Jeong, J-W., Kim, H-J., 2012: Effect of salinity stress on phenolic compounds and carotenoids in buckwheat (*Fagopyrum esculentum* M.) sprout. Food Chemistry 135, 1065–1070.

Liu, J.H., Nada, K., Honda, C., Kitashiba, H., Wen, X.P., Pang, X.M., Moriguchi, T., 2006: Polyamine biosynthesis of apple callus under salt stress: importance of the arginine decarboxylase pathway in stress response. Journal of Experimental Botany 57, 2589-2599.

Ma, Y., Dias, M.C., Freitas, H., 2020: Drought and salinity stress responses and microbe-induced tolerance in plants. Frontiers in Plant Science 11, 591911.

Manan, A., Ayyub, C.M., Pervez, M.A., Ahmad, R., 2016: Methyl jasmonate brings about resistance against salinity stressed tomato plants by altering biochemical and physiological processes. Pakistan Journal of Agricultural Sciences 53, 35-41.

Mersie, W., Singh, M., 1993: Phenolic acids affect photosynthesis and protein synthesis by isolated leaf cells of velvet-leaf. Journal of Chemical Ecology 19, 1293-1301.

Mostafa, H.A.M., Hassanein, R.A., Khalil, S.I., El-Khawas, S.A., El-Bassiouny, H.M.S., El-Monem, A.A.A. 2010: Effect of arginine or putrescine on growth, yield and yield components of late sowing wheat. Journal of Applied Sciences Research 6, 177–183.

Nakano, Y., Asada, K., 1981: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology 22, 867–880.

Nasibi, F., Kalantari, K.M., Barand, A., 2014: Effect of seed pretreatment with L-arginine on improvement of seedling growth and alleviation of oxidative damage in canola plants subjected to salt stress. Iranian Journal of Plant Physiology 5, 1217–1224.

Nejadalimoradi, H., Nasibi, F., Kalantari, K.M., Zanganeh, R., 2014: Effect of seed priming with L-arginine and sodium nitroprusside on some physiological parameters and antioxidant enzymes of sunflower plants exposed to salt stress. Agricultural communications 2, 23–30.

Oba, G., Nordal, I., Stenseth, N.C., Stave, J., Bjora, C.S., Muthondeki, J.K., Bii, W.K.A., 2001: Growth performance of exotic and indigenous tree species in saline soils in Turkana, Kenya. Journal of Arid Environments 47, 499-511.

Patel, S., 2016: *Salicornia*: evaluating the halophytic extremophile as a food and a pharmaceutical candidate. 3 Biotech 6, 104.

Polash, M.A.S., Sakil, A., Hossain, A., 2019: Plants responses and their physiological and biochemical defense mechanisms against salinity: a review. Tropical Plant Research 6, 250–274.

Qados, A.M.S.A., 2010: Effect of arginine on growth, nutrient composition, yield and nutritional value of mung bean plants grown under salinity stress. Nature and Science 8, 30–42.

Qiu, Z., Guo, J., Zhu, A., Zhang, L., Zhang, M., 2014: Exogenous jasmonic acid can enhance tolerance of wheat seedlings to salt stress. Ecotoxicology and Environmental Safety 104, 202–208.

Rady, M.M., Sadak, M.S., El-Lethy, S.R., Abd El-Hamid, E.M., Abdelhamid, M.T., 2015: Exogenous α-tocopherol has a beneficial effect on *Glycine max* (L.) plants irrigated with diluted sea water. The Journal of Horticultural Science and Biotechnology 90, 195–202.

Ramadan, A.A., Abd Elhamid, E.M., Sadak, M.S., 2019: Comparative study for the effect of arginine and sodium nitroprusside on sunflower plants grown under salinity stress conditions. Bulletin of the National Research Centre 43, 118.

Sairam, R.K., Saxena, D.C., 2000: Oxidative stress and antioxidants in wheat genotypes: possible mechanism of water stress tolerance. Journal of Agronomy and Crop Science 184, 55–61.

Sarker, U., Islam, M.T., Oba, S., 2018: Salinity stress accelerates nutrients, dietary fiber, minerals, phytochemicals and antioxidant activity in *Amaranthus tricolor* leaves. PLoS ONE 13, e0206388.

Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M., 1999: Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteau reagent. Methods in Enzymology 299, 152–178.

Sofo, A., Dichio, B., Xiloyannis, C., Masia, A., 2005: Antioxidant defences in olive trees during drought stress: changes in activity of some antioxidant enzymes. Functional Plant Biology 32, 45–53.

Srinivas, N.D., Rashmi, K.R., Raghavarao, K.S.M.S., 1999: Extraction and purification of plant peroxidase by aqueous two-phase extraction coupled with gel filtration. Process Biochemistry 35, 43–48.

Thipyapong, P., Melkonian, J., Wolfe, D.W., Steffens, J.C., 2004: Suppression of polyphenol oxidases increases stress tolerance in tomato. Plant Science 167, 693–703.

Velitcukova, M., Fedina, I., 1998: Response of photosynthesis of *Pisum sativum* to salt stress as affected by methyl jasmonate. Photosynthetica 35, 89-97.

Weckx, J., Clijsters, H., 1997: Zn phytotoxicity induces oxidative stress in primary leaves of *Phaseolus vulgaris*. Plant Physiology and Biochemistry 35, 405-410.

Winicov, I., Bastola, D.R., 1997: Salt tolerance in crop plants: new approaches through tissue culture and gene regulation. Acta Physiologiae Plantarum 19, 435-449.

Wu, H., Liu, X., You, L., Zhang, L., Zhou, D., Feng, J., Zhao, J., Yu, J., 2012: Effects of salinity on metabolic profiles, gene expressions and antioxidant enzymes in halophyte *Suaeda salsa*. Journal of Plant Growth Regulation 31, 332–341.

Zeid, I.M., 2009: Effect of arginine and urea on polyamines content and growth of bean under salinity stress. Acta Physiologiae Plantarum 31, 65–70.