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# Salt stress effects on growth, pigments, proteins and lipid peroxidation in *Salicornia persica* and *S. europaea*

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## Abstract

The effects of NaCl stress on growth, water status, contents of protein, proline, malondialdehyde (MDA), various sugars and photosynthetic pigments were investigated in seedlings of *Salicornia persica* and *S. europaea* grown *in vitro*. Seeds were germinated under NaCl (0, 100, 200, 300, 400, 500 and 600 mM) on Murashige and Skoog medium for 45 d. The shoot growth of both species 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. Proline content in *S. persica* was higher than in *S. europaea* at most NaCl concentrations. Proline, reducing saccharide, oligosaccharide and soluble saccharide contents increased under salinity in both species. In contrast, contents of proteins and polysaccharides reduced in both species under salt stress. MDA content remained close to control at moderate NaCl concentrations (100 and 200 mM) and increased at higher salinities. MDA content in *S. europaea* was significantly higher than *S. persica* at higher salinities. Salt treatments decreased $K^+$ and P contents in seedlings of both species. Significant reduction in contents of chlorophylls and carotenoids due to NaCl stress was also observed in seedlings of both species. Some differences appeared between *S. persica* and *S. europaea* concerning proteins profile. On the basis of the data obtained, *S. persica* is more salt-tolerant than *S. europaea*.

*Additional key words*: halophyte, *in vitro* culture, free proline, MDA, photosynthetic pigment, RWC, salinity, saccharides.

## Introduction

Halophytes are known for their ability to adapt to salinity by altering their energy metabolism (Winicov and Bastola 1997). These plants provide viable organisms for studying the mechanisms they use to handle high salt concentrations (Moghaieb *et al.* 2004). The elucidation of physiological and biochemical mechanisms are critical before trying to introduce genetic and environmental improvements to salt stress (Meloni *et al*. 2003). Salinity affects numerous physiological or biochemical processes, many of which are seen at the cellular level. *In vitro* culture techniques provide controlled, uniform environments to study the salt-stress response of seedlings and undifferentiated callus, thus eliminating complications arising from genetic and morphological variability associated with tissues of whole and mature plants even within the same species (McCoy 1987, Niknam *et al.* 2006). Adaptation of halophytes to salinity is associated with osmotic adjustment that leads to the accumulation of several organic solutes, such as free proline and sugars (Bohnert *et al*. 1995). Plants that are subjected to environmental stress often suffer oxidative damage (Scandalios 1993). Plants protect cells and subcellular systems from the effects of reactive oxygen species (ROS) by enzymes such as superoxide dismutase, catalase, peroxidase, glutathione reductase, polyphenol oxidase and non-enzymic antioxidants such as ascorbate and glutathione (Agarwal and Pandey 2004). Malondialdehyde (MDA) content, a product of lipid peroxidation, has been considered as an indicator of oxidative damage. Thus cell membrane stability has widely been utilized to differentiated sat-tolerant and salt-sensitive plants (Shalata *et al*. 2001, Hernandez and Almansa 2002,

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*Abbreviations*: Car - carotenoid; Chl - chlorophyll; MDA - malondialdehyde; MS medium - Murashige and Skoog medium; RWC - relative water content; WC - water content.
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Meloni *et al*. 2003, Sairam *et al*. 2005).

*Salicornia persica* and *S. europaea* are annual and succulent euhalophytes belonging to *Chenopodiaceae*. *S. persica* is distinguished from *S. europaea* by having ascending habit, verticillate inflorescence branches and reversed pentagonal central flowers that are truncated at the apex and reach to the upper segment and the species is tetraploid (2n=36) (Akhani 2003). Under seawater irrigation, the seeds of *S. europaea* have an oil content of 28 % and protein content of 30.2 % (O' Leary *et al*. 1985). Unsaturated fatty acid constituted a large percentage of seed oil in *S. europaea* (Zhao and Feng 2001). So, *S. europaea* and *S. persica* could be competitive potential oil seed crops as well.

In order to look into salinity stress induced biochemical and physiological changes and to elucidate adaptive mechanisms, we investigated the status of growth and contents of saccharides, free proline, protein and MDA in seedlings of *S. persica* and *S. europaea* grown under NaCl. Using these species as models, this study examined for the first time the differences in the salt tolerance mechanisms between two halophytic species, *S. persica* and *S. europaea*, with the aim of improving the salt tolerance of non-halophytic plants.

## Materials and methods

Seeds of *Salicornia persica* Akhani and *Salicornia europaea* L. were collected during autumn 2005 from inland salt marshes in Fars and Azarbayejan provinces of Iran. Seeds were separated from the inflorescence and stored at 4 °C. Then they were surface sterilized in 10 % (v/v) sodium hypochlorite solution containing a few drops of *Tween 20* for 5 min, followed by 3 times washes with sterile distilled water. Seeds were germinated and maintained for 45 d on Murashige and Skoog (1962, MS) medium containing 0, 100, 200, 300, 400, 500 and 600 mM NaCl under 16-h photoperiod (white fluorescent lamps, irradiance of 33 μmol $m^{-2}$ $s^{-1}$) and temperature of 25 ± 2 °C.

Leaf relative water content (RWC) was estimated according to Wheatherley (1950) and calculated as: RWC = [(f.m. - d.m.)/(saturated mass - d.m.)] × 100. Saturated mass was determined by keeping seedlings in water for 24 h, d.m. by their drying in hot air oven (50 °C) for 48 h. The osmotic potential (Ψs) of cell sap was measured using vapor pressure osmometer (model *VAPOR 5520*, *Wescor*, Logan, USA).

For determination of saccharide content, 50 mg of dry powder was extracted using 10 $cm^3$ of ethanol:distilled water (8:2; v/v), and supernatant was collected after twice centrifugation at 1 480 *g*. The residue from ethanol extraction was subsequently used for polysaccharide extraction by boiling water (Niknam *et al*. 2004). Total saccharide content was estimated by the method of Dubois *et al*. (1956). Reducing saccharides were quantified according to Nelson (1944). Oligosaccharide content was obtained from difference between soluble and reducing saccharide content.

Free proline content was determined according to Bates *et al*. (1973) using L-proline as a standard. High-speed centrifuge (*Beckman J2-21M*, Palo Alto, USA) and UV-visible spectrophotometer (*Shimadzu UV-160*, Tokyo, Japan) with 10 mm matched quartz cells were used for centrifugation of the extracts and determination of the absorbance, respectively. For determination of protein content, 500 mg fresh seedling was homogenized in a chilled (4 °C) mortar using a 50 mM Tris-HCl buffer (pH 7.0) containing 10 mM EDTA, 2 mM $MgSO_4$, 20 mM dithiotreitol, 10 % (v/v) glycerol and 2 % (m/v) polyvinylpyrrolidone. After centrifugation at 13 000 *g* for 45 min at 4 °C, the supernatant was filtered and then transferred to Eppendorf tubes and the sample kept on ice at 4 °C. A portion of eluent was stored at -70 °C. Total protein content was measured by the spectrophotometric method of Bradford (1976) using bovine serum albumin (BSA) as the standard.

Discontinuous sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (1970) with 12 % acrylamide gels. For detection of proteins, gels were stained with 0.03 % Coomassie Briliant Blue G250. A vertical electrophoresis apparatus (*model LKB*, Bromma, Sweden) was used. The electrophoretic run was carried out with 120 mV (30 mA) per plate towards the cathode.

The lipid peroxidation was measured in terms of thiobarbituric acid reactive substances (TBARS), following the method of Heath and Packer (1968). The seedlings (0.5 g) were homogenized in 5 $cm^3$ of 0.1 % (m/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10 000 *g* for 20 min. To 1 $cm^3$ aliquot of the supernatant, 4 $cm^3$ of 0.5 % thiobarbituric acid (TBA) in 20 % TCA was added. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10 000 *g* for 15 min, the absorbance of the supernatant was recorded at 532 and 600 nm. The value for non-specific absorption at 600 nm was subtracted. The concentration of MDA was calculated using coefficient of absorbance 155 $mM^{-1}$ $cm^{-1}$.

The $K^+$ and $Na^+$ contents were determined by flame photometer (*Jenway PFP7*, Essex, UK) and inorganic P content by spectrophotometer (*Shimadzu UV-160*). Before measurement, 50 mg of dried sample was digested in 20 $cm^3$ of 70 % (v/v) perchloric acid.

Chlorophylls (Chl *a* and Chl *b*) and carotenoids were extracted by 80 % aceton and quantified spectrophotometrically according to Lichtenthaler and Wellburm (1983).

The data determined in triplicate were analyzed by analysis of variance (*ANOVA*) using *SPSS* (version *9.05*). The significance of differences was determined according to Duncan's multiple range test (DMRT). *P* values < 0.05 are considered to be significant.

## Results and discussion

Seedling fresh and dry masses in *Salicornia persica* increased significantly at 100 mM NaCl then returned to the control values at medium salinities (200 and 300 mM NaCl) and decreased at higher concentrations (Fig. 1*A*,*B*).

The present data agree with previous data reported on *Salicornia bigelovii* (Ayala and O'Leary 1995),

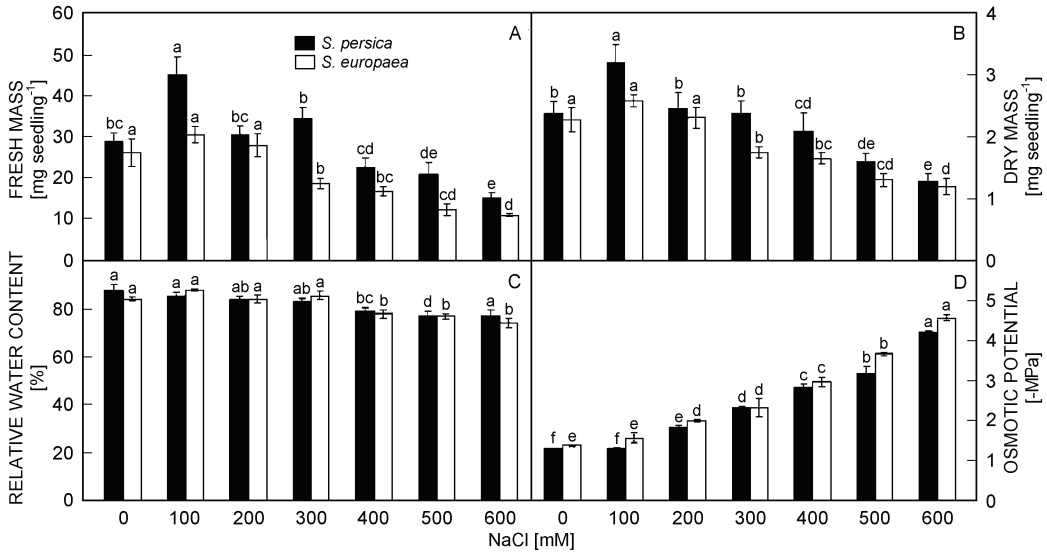


Fig. 1. Fresh mass (*A*), dry mass (*B*), RWC (*C*), and osmotic potential (*D*) in seedlings of *S. persica* and *S. europaea* under NaCl stress. Each value represents the mean of three replicates. *Bars* indicate SE. Different letters over the bars indicate significant differences ($P < 0.05$).

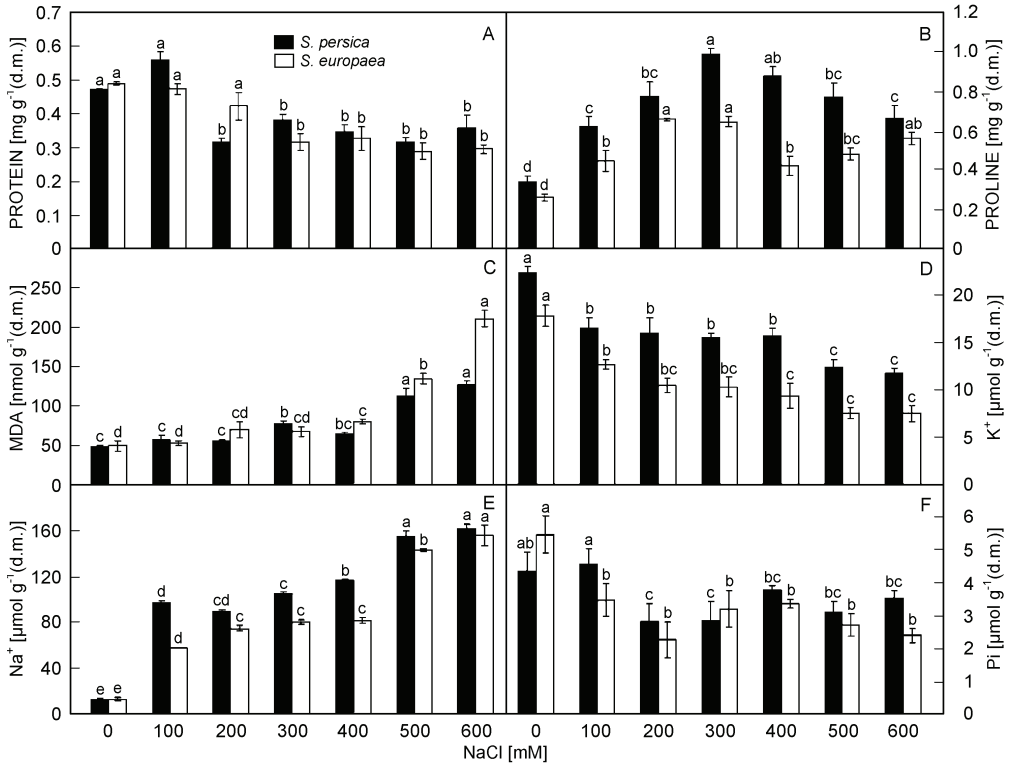


Fig. 2. Contents of protein (*A*), proline (*B*), MDA (*C*), $K^+$ (*D*), $Na^+$ (*E*) and $P_i$ (*F*) in seedlings of *S. persica* and *S. europaea* under NaCl stress. Means ± SE of three replicates. Different letters indicate significant differences ($P < 0.05$).

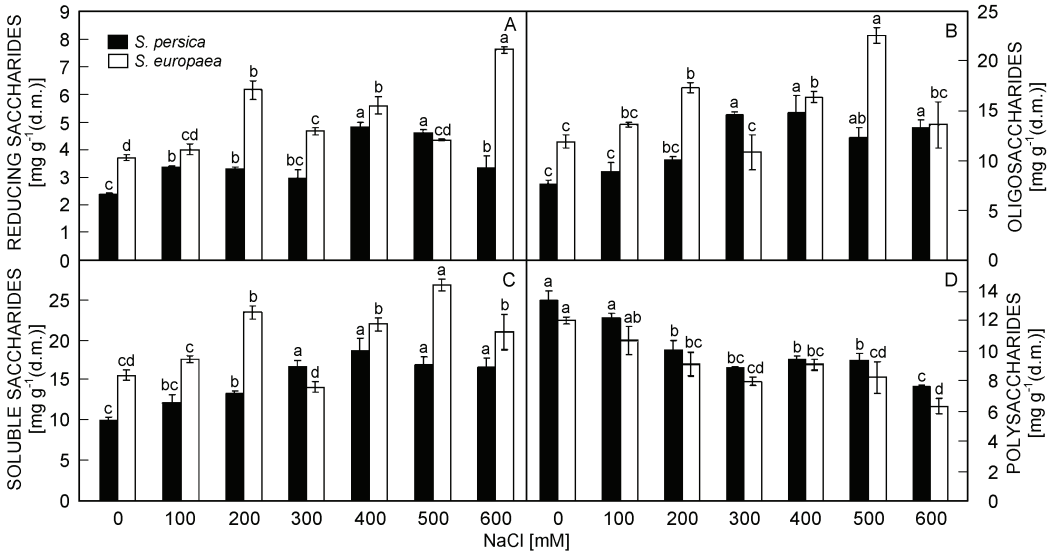


Fig. 3. Reducing saccharide (*A*), oligosaccharide (*B*), soluble saccharide (*C*) and polysaccharide (D) contents in seedlings of *S. persica* and *S. europaea* under NaCl stress. Means ± SE of three replicates. Different letters indicate significant differences ($P < 0.05$).

*Salicornia europaea* and *Suaeda maritima* (Moghaieb *et al*. 2004) in which salt treatment at low levels improves plant growth. The seedling of both species is still able to grow in the presence of 300 mM NaCl and remains alive when confronted to 600 mM NaCl that is a higher dose than salt concentration of sea water. Similar results were previously reported in other halophytic species (Ajmal Khan *et al*. 2000, Amor *et al*. 2005, Heidari-Sharifabadi and Mirzaie-Nadoshan 2006). Root length in *S. europaea* decreased significantly with an increase in salinity up to 200 mM NaCl then remained constant at higher concentrations. Then, based on the analyses of fresh and dry mass, *S. persica* was more salt tolerant than *S. europaea*.

Significant reduction of $K^+$ and $P_i$ contents under NaCl salinity was concomitant with a significant $Na^+$ accumulation in seedlings of both species (Fig. 2*D*,*F*). At 600 mM NaCl, $Na^+$ content in seedlings of *S. persica* and *S. europaea* amounted to 1182.9 % and 1087.7 % of the control, respectively. To maintain an osmotic gradient for the uptake of water from medium, many halophytic plants accumulate inorganic ions to a concentration equal to or greater than that of the surrounding root solution (Bradley and Morris 1991).

NaCl increased the contents of reducing saccharides, oligosaccharides and soluble saccharides in both species comparing to that of control. In contrast, polysaccharide content decreased under NaCl salinity in both species (Fig. 3*A*-*D*). The accumulation of soluble sugars in response to salinity and water stress was well documented (*e.g.* Binzel *et al.* 1989, Dubey and Singh 1999). According to our results this accumulation is more prominent in *S. europaea* than that *S. persica*. It is believed that under salinity stress accumulation of sugars along with other compatible solutes contribute to osmotic adjustment (*e.g.* Bohnert *et al*. 1995) and/or stabilization of the cell membranes and protein (Sanchez *et al*. 1998).

Chl *a* and Chl *b* and carotenoid contents in both species decreased remarkably under NaCl stress (Fig. 4). The loss of chlorophylls under salt stress could be related

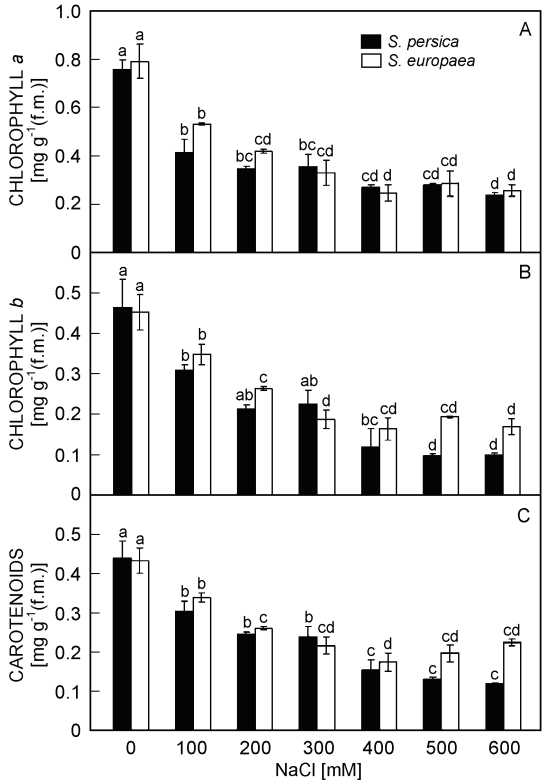


Fig. 4. Chl *a* (*A*), Chl *b* (*B*) and Car (*C*) contents in seedlings of *S. persica* and *S. europaea* under NaCl stress. Means ± SE of three replicates. Different letters indicate significant differences ($P < 0.05$).

to photoinhibition or ROS formation (Kato and Shimizu 1985), as demonstrated by the increased lipid peroxidation. Salt-induced decreases in photosynthetic pigments have been reported previously in various species (*e.g.* Parida *et al*. 2002, Meloni *et al*. 2003).

According to SDS-PAGE analysis, proteins patterns in seedlings of both species under different concentrations of NaCl were not identical and the differences were both quantitative and qualitative (Fig. 5*A,B*). In *S. persica*, polypeptides of 16, 26, 27, 28 and 31 kDa decreased under NaCl treatments while a 32 kDa polypeptide was remained unchanged (Fig. 5*A*). In *S. europaea*, a 26 kDa polypeptide was remained unchanged and several polypeptides of 27, 28, 31, 32 and 54 kDa decreased under NaCl treatments. These observations suggest the possible involvement of these proteins for osmotic adjustment under salt stress. In accordance to our results, Parida *et al.* (2004) and Hassanein (1999) reported the reduction in several protein contents in *Bruguiera parviflora* and *Arachis hypogaea*, respectively. In the present study, a 31 kDa protein disappeared at 200 mM NaCl in *S. persica.* The disappearance of proteins in response to NaCl salinity has been observed in *Prosopsis* (Munoz *et al.* 1997), wheat (Elshintinawy and Elshourbagy 2001) and *Bruguiera parviflora* (Parida *et al.* 2004). In this research 200 mM NaCl and higher induced a 39 kDa protein in *S. persica.* These results confirmed our previous data on *Trigonella* (Niknam *et al.* 2006). To identify these polypeptides would have contributes to understanding the intracellular mechanism of *Salicornia* response to salinity.

In summary, our results showed that *S. persica* and *S. europaea* exhibit growth and other properties entirely consistent with those of other halophytes. They grow rapidly at moderate salt concentrations and can survive at extreme salinities, including near seawater concentrations. The present study showed that salinity triggered some solutes (proline and soluble saccharidess) and inorganic ions ($Na^+$) accumulation. *S. persica*, showed a higher degree of accumulation of proline and lower level of soluble saccharides than the *S. europaea*. Lower level of lipid peroxidation in *S. persica* may, at least in part, explain the greater tolerance of *S. persica* comparing to *S. europaea*.

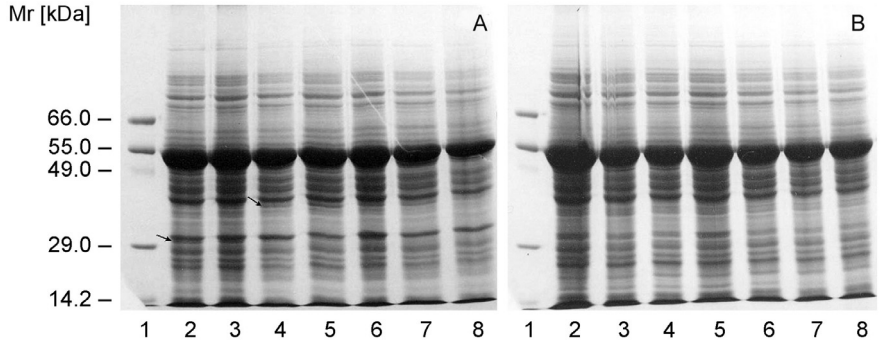


Fig. 5. SDS-PAGE pattern of proteins in seedlings of *S. persica* (A) and *S. europaea* (B) under different concentrations of NaCl: Molecular mass markers (1), and seedlings at 0, 100, 200, 300, 400, 500 and 600 mM NaCl, (2 to 8). *Arrows* indicate some of the affected bands.

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