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Salt tolerance of a cash crop halophyte *Suaeda fruticosa*: biochemical responses to salt and exogenous chemical treatments

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Abstract *Suaeda fruticosa* Forssk is a leaf succulent obligate halophyte that produces numerous seeds under saline conditions. Seeds are a good source of high quality edible oil and leaves are capable of removing substantial amount of salt from the saline soil besides many other economic usages. Little is known about the biochemical basis of salt tolerance in this species. We studied some biochemical responses of *S. fruticosa* to different exogenous treatments under non-saline (0 mM), moderate (300 mM) or high (600 mM) NaCl levels. Eight-week-old seedlings were sprayed twice a week with distilled water, hydrogen peroxide (H_2O_2, 100 μM), glycine betaine (GB, 10 mM), or ascorbic acid (AsA, 20 mM) for 30 days. At moderate (300 mM) NaCl, leaf Na^+, Ca^{2+} and osmolality increased, along with unchanged ROS and antioxidant enzyme activities, possibly causing a better plant growth. Plants grew slowly at 600 mM NaCl to avoid leaf Na^+ buildup relative to those at 300 mM NaCl. Exogenous application of distilled water and H_2O_2 improved ROS scavenging mechanisms, although growth was unaffected. ASA and GB alleviated salt-induced growth inhibition at 600 mM NaCl through enhancing the antioxidant defense system and osmotic and ion homeostasis, respectively.

Keywords Antioxidant · Exogenous treatment · Oxidative stress · Halophyte · Reactive oxygen species

Abbreviations

APX	Ascorbate peroxidase
AsA	Ascorbic acid/ascorbate
CAT	Catalase
DW	Dry weight
FW	Fresh weight
GB	Glycinebetaine
GPX	Guaiacol peroxidase
GR	Glutathione reductase
GRX	Glutaredoxins
GSH	Glutathione
GST	Glutathione-*S*-transferase
MDA	Malondialdehyde
OPP	Oxidative pentose phosphate pathway
OW	Organic weight
PAR	Photosynthetically active radiation
POD	Peroxidases
Pro	Proline
PRX	Peroxiredoxins
ROS	Reactive oxygen species
SOD	Superoxide dismutase

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Introduction

Suaeda fruticosa Forssk is distributed in coastal and inland saline communities of Pakistan and throughout the saharo-sindian and southern irano-turanian regions (http://www.efloras.org/florataxon.aspx?flora_id=5&taxon_id=242100197). This species is highly polymorphic with various forms (planar, terete or spheroid) and shades of succulent leaves. Economic usages of this plant include good forage for camels (Towhidi et al. 2011), high quality edible oil from seeds (Weber et al. 2007), domestic soap from burnt leaves (Freitag et al. 2001) and antiophthalmic, hypolipidaemic and hypoglycemic effects of different plant parts (Bennani-Kabachi et al. 1999; Benwahhoud et al. 2001; Chopra et al. 1986). Cultivation of *S. fruticosa* could help in bioremediation and reclamation of soils contaminated with toxic metals (Bareen and Tahira 2011) and salinity (Khan et al. 2009). The plant can survive high (1,000 mM) NaCl concentrations and grows optimally in the range of 200–400 mM NaCl (Khan et al. 2000), by sequestering large amounts of vacuolar NaCl and cytoplasmic glycinebetaine (GB), the latter being a characteristic feature of the family Amaranthaceae. However, other underlying mechanisms of salt tolerance of *S. fruticosa* are not known.

Soil salinity disrupts water uptake and ion equilibrium of plants, eventually leading to oxidative damage to membrane lipids, proteins and nucleic acids (Munns and Tester 2008; Zhu 2001). High salt stress limits CO_2 fixation resulting in over-reduced photosynthetic machinery, which accelerates the production of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) (Miller et al. 2010). Halophytes are reported to keep the cellular levels of these potentially damaging ROS within a narrow, functionally important range under optimum growing conditions by utilizing a coordinated antioxidant system consisting of enzymes like superoxide dismutases (SOD), catalases (CAT) and peroxidases (POD) and non-enzymatic antioxidants like ascorbate (AsA) and glutathione (GSH) (Jithesh et al. 2006; Shabala and Mackay 2011). Therefore, at the whole plant level, a strong antioxidant defense system along with efficient ion regulation, production of compatible solutes, and the maintenance of photosynthesis is attributed to salt tolerance in halophytes (Flowers and Colmer 2008; Guan et al. 2011; Jithesh et al. 2006; Shabala and Mackay 2011; Song et al. 2006). However, these defense mechanisms would become inadequate under high saline conditions leading to growth inhibition and/or death (Flowers and Colmer 2008; Jithesh et al. 2006; Munns and Tester 2008).

Exogenous application of different chemicals such as glycinebetaine (Abbas et al. 2010; Demiral and Türkan 2004; Raza et al. 2007), ascorbic acid (Athar et al. 2008; Salama 2009; Shalata and Neumann 2001) and hydrogen peroxide (Gondim et al. 2010; Gong et al. 2001; Li 2011; Neto et al. 2005; Wahid et al. 2007) is reported to improve salt tolerance of many crop plants, however, such studies are not reported for halophytes. Plant responses to different exogenous chemicals are quite variable and inconclusive. Such studies involve spray of aqueous solutions of different chemicals and any improvement in salt tolerance might be a consequence of water rather than the chemical itself. Therefore, use of water spray as a positive control in such experiments is important.

We examined growth and some biochemical responses of *S. fruticosa* under saline conditions with and without exogenous spray of growth promoting chemicals and distilled water to answer the following questions: 1) how do biochemical responses of *S. fruticosa* in moderate NaCl treatment differ from those in high salt stress? 2) could exogenous application of different growth regulating chemicals improve salt tolerance of *S. fruticosa*? 3) if foliar application of hydrogen peroxide is damaging to the plants? and 4) whether foliar spray of distilled water has some role in improving salt tolerance of our test species?

Materials and methods

Sample collection

Mature inflorescence of *S. fruticosa* were collected from a salt-flat located near low dunes of Hawks Bay, Karachi, Pakistan (24°52′21.87″N, 66°51′24.58″E, 17 ft elevation, 1.5 km away from the sea front) in plastic bags and transported to the laboratory. Seeds were separated from the inflorescence, surface sterilized with 1 % sodium hypochlorite for 1 min, followed by thorough rinsing with distilled water and air-drying. Seeds were stored at room temperature in dry clear petri plates.

Growth conditions

Seedlings were raised in 12 cm dia. plastic pots on sandy soil and sub-irrigated with half strength Hoagland's nutrient solution in a green net house (PAR ~600 µmol m^{-2} s^{-1}) under semi-ambient environmental conditions. After 8 weeks NaCl (0, 300 and 600 mM) was gradually introduced at the rate of 150 mM NaCl after 48 h intervals to avoid osmotic shock in such a way that all final salinity concentrations were achieved on the same day. Fresh water was added daily to compensate for loss due to evaporation. Ascorbic acid (AsA, 20 mM), glycinebetaine (GB, 10 mM), hydrogen peroxide (H_2O_2, 100 µM) and distilled water each with 0.1 % Tween-20 were sprayed 7 days after achieving final salinity concentrations, on the leaves of

plants till dripping at 16:30 h twice a week till harvest. Concentrations of chemicals sprayed were determined in preliminary trials. Unsprayed plants served as control for studying the effect of foliar sprays. There were five plants per pot and four pots per treatment. Plants were harvested 4 weeks after final salinity concentrations achieved.

Growth parameters

Fresh weight (FW) of plants was recorded immediately after harvest. Dry weight (DW) was determined after drying vegetative parts for 48 h in a forced-draft oven at 60 °C. Tissue water was calculated by subtracting dry biomass from fresh biomass. Ash was determined by igniting the dried biomass in furnace at 550 °C for 6 h. Plant organic weight (OW) was calculated by subtracting ash from the dry biomass.

Leaf osmolality

Osmolality of the leaf sap was determined using a vapor pressure osmometer (VAPRO 5520, Wescor Inc, USA).

Oxidative stress markers

Malondialdehyde (MDA) contents were quantified as an estimate of lipid peroxidation with slight modifications of method reported by Heath and Packer (1968). Fresh leaves (0.5 g) were homogenized in 5 mL of 1 % ice-cold trichloroacetic acid in ice-chilled mortar and pestle. The homogenate was centrifuged at 12,000×*g* for 20 min at 4 °C. Half milliliter of supernatant was mixed with 0.5 mL of 20 % (w/v) trichloroacetic acid containing 0.5 % 2-thiobarbituric acid heated at 95 °C for 30 min in a shaking water bath. The reaction was terminated in an ice bath followed by centrifugation at 12,000×*g* for 10 min at 4 °C. Absorbance was measured at 532 and 600 nm ($\varepsilon = 155\ mM^{-1}\ cm^{-1}$).

Endogenous H_2O_2 was determined by the method of Loreto and Velikova (2001) with minor modifications. Fresh leaves (0.5 g) were homogenized in 5 mL of 1 % ice-cold trichloroacetic acid with ice-chilled mortar and pestle. The homogenate was centrifuged at 12,000×*g* for 20 min at 4 °C. Subsequently 0.75 mL of supernatant was mixed with 0.75 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1.5 mL of 1 M KI. Absorbance was recorded at 390 nm and H_2O_2 levels were calculated with the help of a standard curve.

Antioxidant enzymes

Antioxidant enzymes were extracted according to Polle et al. (1994). Extracts were stored at −80 °C prior to antioxidant enzyme assays which were carried out at 25 °C. Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined by measuring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm (Beauchamp and Fridovich 1971). Catalase (CAT, EC 1.11.1.6) activity was assayed by monitoring the decomposition of H_2O_2 ($\varepsilon = 39.4\ mM\ cm^{-1}$) at 240 nm for 1 min (Abey 1984). Guaiacol peroxidase (GPX, EC 1.11.17) activity was measured by monitoring the increase in absorbance due to tetraguaiacol formation ($\varepsilon = 26.6\ mM\ cm^{-1}$) at 470 nm (Tatiana et al. 1999). Ascorbate peroxidase (APX, EC 1.11.1.11) activity ($\varepsilon = 2.8\ mM\ cm^{-1}$) was examined according to Nakano and Asada (1981) by recording the decrease in absorbance due to the oxidation of ascorbic acid at 290 nm. Glutathione reductase (GR, EC 1.6.4.2) activity was quantified by measuring the oxidation of NADPH ($\varepsilon = 6.2\ mM\ cm^{-1}$) at 340 nm (Foyer and Halliwell 1976). Protein was estimated according to Bradford (1976) and enzymes activities were expressed as units per milligrams of the protein.

Osmoprotectants and antioxidants

Hot water extracts were prepared by boiling ground dry leaves in 10 mL of distilled water for 1 h. Free proline (Pro) was quantified by the method of Bates et al. (1973). Total soluble sugars were estimated in hot water extract with anthrone method (Ludwig and Goldberg 1956; Yemm and Willis 1954). Reduced glutathione (GSH) was extracted from fresh leaves (0.5 g) by homogenization in 5 mL of 1 % ice-cold trichloroacetic acid with the help of ice-chilled mortar and pestle. Homogenate was centrifuged at 12,000×*g* for 20 min at 4 °C. Supernatant was used to quantify GSH according to Guri (1983). Ascorbic acid (AsA) was determined by the method of Luwe et al. (1993) in the TCA extracts.

Cations

Cations (Na^+ and Ca^{2+}) were determined in hot water extracts with the help of atomic absorption spectrometer (Perkin Elmer AA-700).

Statistical analyses

Data were analyzed using SPSS version 11.0 for windows (SPSS 2001). Analysis of variance (ANOVA) was used to test significance of the treatments on different growth parameters of the test species. Bonferroni test was carried out to determine if significant ($P < 0.05$) differences existed among means.

Results

Growth parameters

NaCl had a significant effect on the FW ($F = 71.7$; $P < 0.0001$), DW ($F = 57.1$; $P < 0.0001$), ash ($F = 65.7$; $P < 0.0001$), tissue water ($F = 45.6$; $P < 0.0001$) and OW ($F = 53.1$; $P < 0.0001$) of the shoots of *S. fruticosa*. DW and OW increased at moderate (300 mM) NaCl treatment compared to other NaCl treatments (Fig. 1). Growth was substantially reduced at 600 mM NaCl. Exogenous application of AsA and GB significantly ($P < 0.05$) improved growth in all salinity treatments (Fig. 1).

Leaf osmolality

Leaf osmolality significantly ($F = 121.9$; $P < 0.0001$) increased with increasing NaCl concentrations and also in all exogenous treatments ($P < 0.05$) (Fig. 2a).

Cations

Leaf Na^+ increased significantly ($P < 0.05$) with the introduction of NaCl, however, it remained unchanged between 300 and 600 mM (Fig. 2b). It was decreased by exogenous treatments with water and H_2O_2 (600 mM NaCl), GB (300 and 600 mM NaCl) and AsA (300 mM NaCl) (Fig. 2b). Leaf Ca^{2+} peaked significantly ($P < 0.05$) at 300 mM NaCl with comparable values in 0 and 600 mM NaCl (Fig. 2c). All exogenous treatments increased Ca^{2+} in leaves except AsA in 300 mM NaCl and water at all salinities (Fig. 2c).

Oxidative stress markers

A significant increase in MDA ($F = 4.2$; $P < 0.001$) and endogenous H_2O_2 ($F = 3.8$; $P < 0.01$) was observed with increases in NaCl concentration (Fig. 3a, b). Exogenous treatments (except H_2O_2) caused a significant reduction in both MDA and endogenous H_2O_2 at high NaCl concentrations (Fig. 3a, b).

Antioxidant enzymes

Activity of SOD in high NaCl significantly ($P < 0.05$) decreased (Fig. 4a), GPX activity increased ($P < 0.05$) (Fig. 4c), whereas CAT and APX activities remained unchanged (Figs. 4b, 5d). GR activity increased only in 300 mM (Fig. 4e). SOD activity increased in most exogenous applications in all salt treatments except water and GB spray under non-saline conditions (Fig. 4a). CAT activity was higher in all exogenous treatments, except AsA spray in 0 and 600 mM NaCl and GB spray in 0 mM

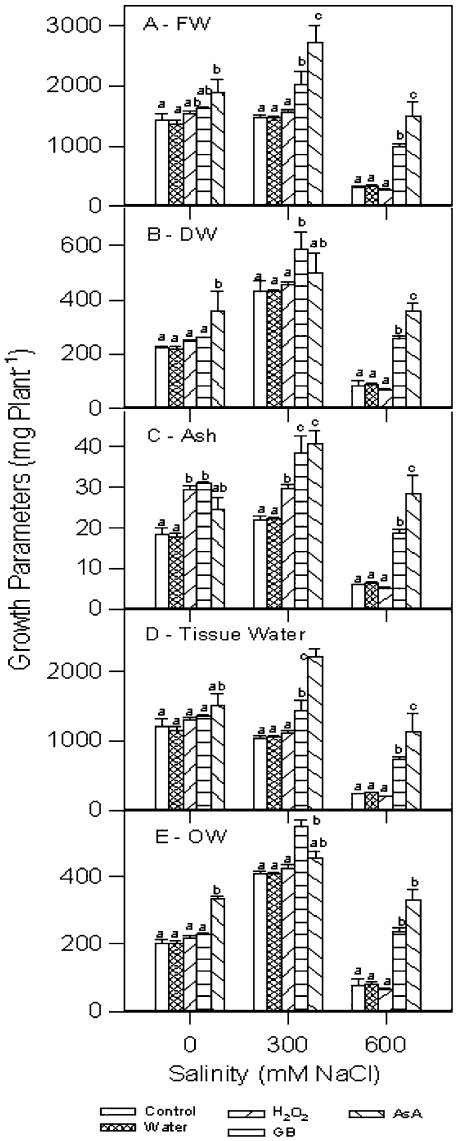

Fig. 1 Shoot growth of *S. fruticosa* in different NaCl concentrations with and without exogenous treatments. *Bars* represent mean ± SE. *Bars* within a salt treatment with *same letter* are not significantly different ($P < 0.05$; Bonferroni test)

NaCl (Fig. 4b). H_2O_2 spray significantly ($P < 0.05$) increased GPX activity in 0 and 600 mM NaCl (Fig. 4c). Most exogenous treatments improved APX and GR activities at all salt treatments (Fig. 4d, e).

Osmoprotectants and antioxidants

Proline (Pro) did not change among salt treatments (Fig. 5a). It was, however, increased in GB- and AsA-treated plants in 300 mM NaCl and by water and H_2O_2 in 600 mM NaCl (Fig. 5a). Although soluble sugar content did not change between 0 and 300 mM NaCl, it substantially ($F = 4.5$; $P < 0.001$) increased at 600 mM NaCl (Fig. 5b). All

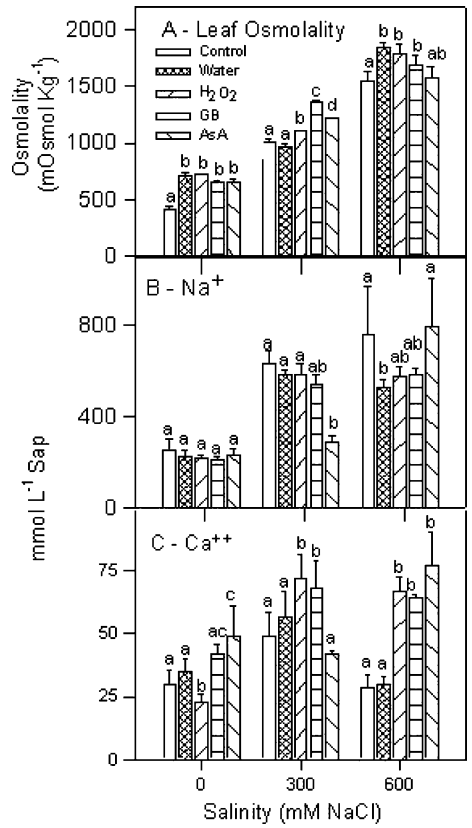

Fig. 2 Leaf osmolality, Na^+ and Ca^{2+} levels in *S. fruticosa* in different NaCl concentrations with and without exogenous treatments. *Bars* represent mean ± SE. *Bars* within a salt treatment with *same letter* are not significantly different ($P < 0.05$; Bonferroni test)

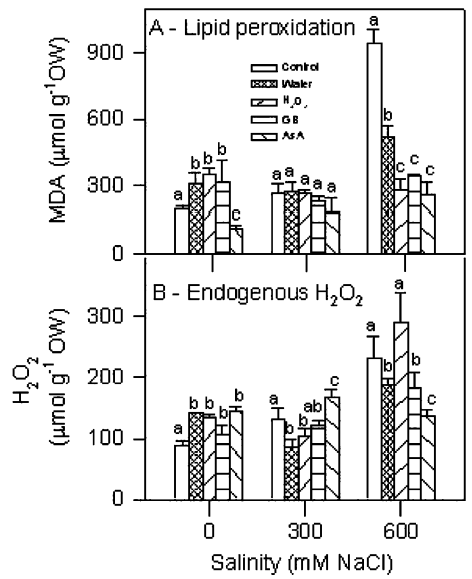

Fig. 3 MDA and endogenous H_2O_2 content in *S. fruticosa* leaves in different NaCl concentrations with and without exogenous treatments. *Bars* represent mean ± SE. *Bars* within a salt treatment with *same letter* are not significantly different ($P < 0.05$; Bonferroni test)

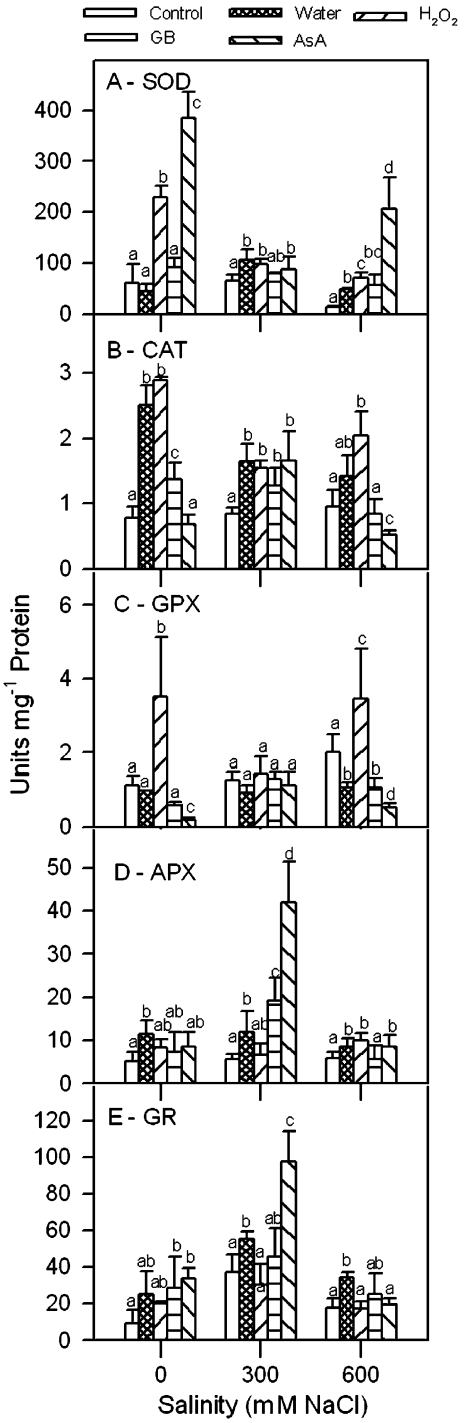

Fig. 4 Activities of various antioxidant enzymes in *S. fruticosa* leaves in different NaCl concentrations with and without exogenous treatments. *Bars* represent mean ± SE. *Bars* within a salt treatment with *same letter* are not significantly different ($P < 0.05$; Bonferroni test)

exogenous treatments increased leaf soluble sugars regardless of the NaCl concentrations used except for GB at 600 mM NaCl (Fig. 5b). GSH ($F = 3.2$; $P < 0.01$) and AsA ($F = 3.5$; $P < 0.01$) increased significantly with increasing

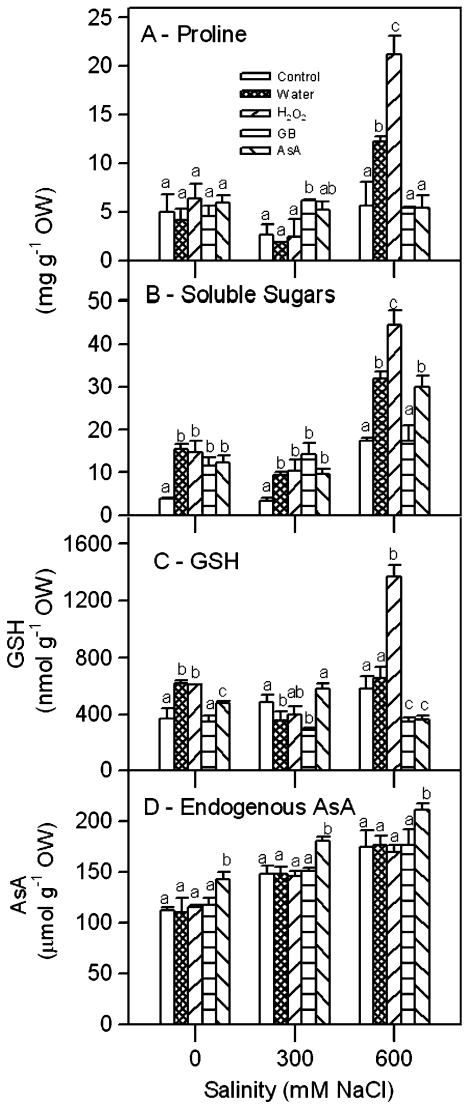

Fig. 5 Concentration of various osmoprotectants/antioxidants in *S. fruticosa* leaves in different NaCl concentrations with and without exogenous treatments. *Bars* represent mean ± SE. *Bars* within a salt treatment with *same letter* are not significantly different ($P < 0.05$; Bonferroni test)

NaCl concentrations (Fig. 5c, d). Higher GSH was observed in H_2O_2-sprayed plants at 600 mM NaCl (Fig. 5c). Higher endogenous AsA was found in AsA-sprayed plants in all salinity treatments (Fig. 5d).

Discussion

Suaeda fruticosa showed optimal growth at 300 mM NaCl in our experimental conditions, which is in accordance with a previous report on same species in a growth chamber (Khan et al. 2000). Species of genus *Suaeda* are reported to be obligate halophyte because their optimal growth is obtained in the presence of salinity like *S. salsa*

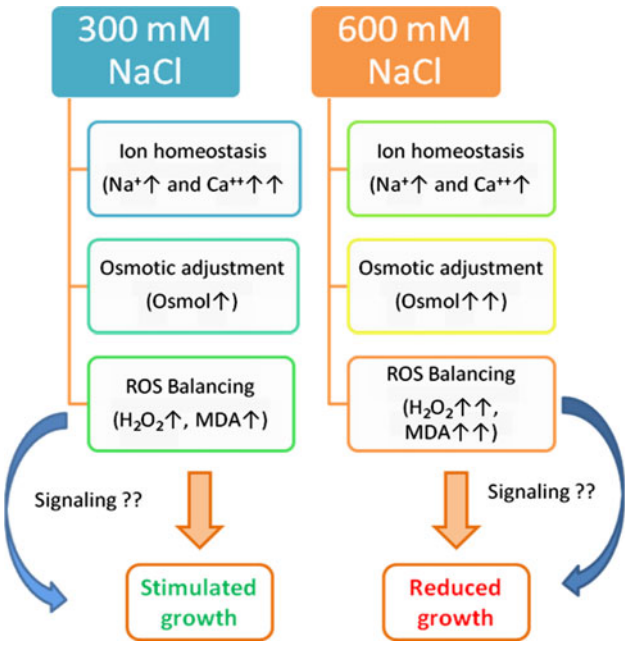

Fig. 6 Hypothetical model describing growth of the *S. fruticosa* under moderate and high NaCl treatments

(100–200 mM NaCl, Song et al. 2009) and *S. maritima* (170–340 mM NaCl; Flowers 1972). Leaf osmolality (1,003 mOsmol Kg^{-1}; ca. −2.4 MPa) of *S. fruticosa* when grown under 300 mM (ca. −1.38 MPa) NaCl appears enough to attain osmotic adjustment. Slightly higher H_2O_2 and MDA at moderate salt stress (300 mM NaCl) perhaps due to unchanged activities of antioxidant enzymes such as APX, GPX and CAT might be involved in activation of stress-responsive metabolism such as H_2O_2-mediated Na^+ homeostasis under saline conditions (Sun et al. 2010). Increase in Ca^{2+} at moderate salt stress also appears to have some role in optimum growth of test species. Sun et al. (2010) reported that Ca^{2+} is involved in H_2O_2-mediated Na^+ homeostasis. Moreover, exogenous application of H_2O_2 also increased Ca^{2+} in leaves of *S. fruticosa* compared to unsprayed leaves, supporting a possible H_2O_2-mediated Ca^{2+} uptake for Na^+ homeostasis at cellular/tissue level. This might be a cause for optimum growth of our species despite a twofold increase in Na^+ at 300 mM NaCl. Hydrogen peroxide is reported to mediate *sos1* mRNA stability in *Arabidopsis* (Chung et al. 2008) and enhanced Na^+/H^+ exchange in *Populus euphratica* under NaCl-treatment in the presence of Ca^{2+} (Sun et al. 2010). In addition, increased GR activity in 300 mM NaCl could be a source for providing $NADP^+$ to oxidative pentose phosphate pathway (OPP), which is involved in a number of growth and developmental processes (Butt and Beevers 1961; Dalling et al. 1972; Kruger and Schaewen 2003; Rees 1985). Reduced glutathione produced due to increased GR activity in 300 mM NaCl might be

transported from leaves to other parts of the plant as reported by Foyer et al. (2001), or it may possibly be consumed in glutathione-dependent peroxide metabolism by glutathione-*S*-transferase (GST), glutaredoxins (GRX) and peroxiredoxins (PRX) for the removal of ROS or may be involved in GSH-linked signaling mechanisms such as S-glutathionylation (Foyer and Noctor 2011). In addition, $NADP^+$ produced by the activity of GR may also decrease over-reduction of PS I. These processes might be involved in optimal growth of *S. fruticosa* in 300 mM NaCl treatment.

Reduced growth in high NaCl treatment (600 mM NaCl; ca. −2.76 MPa) along with sustained high leaf osmolality (1545 mOsmol kg^{-1}; ca. −3.8 MPa), sugars, AsA and GSH appears to be an adaptive response. Higher GPX activity in 600 mM NaCl also supports this assumption, as this enzyme is also associated with growth reduction by either cell wall hardening (Cavalcanti et al. 2004; Dionisio-Sese and Tobita 1998) or destruction of growth-promoting phytohormone IAA (Salin 1987). Reduced GR activity might decrease $NADP^+$ supply to oxidative pentose phosphate pathway (OPP), which supports a number of growth and developmental processes. A 200 % increase in external NaCl from 300 to 600 mM, showed no significant change in leaf Na^+ contents, indicating a tight Na^+ regulation. Similarly, a fivefold increase (from 100 mM to 500 mM) in external salt concentrations resulted in a 50 % increase in the sap Na^+ of *Chenopodium quinoa* probably by a strict control of xylem Na^+ loading or an efficient Na^+ removal from leaves (Hariadi et al. 2011). Salt excretion by stomatal guttation or by cuticular diffusion in the absence of salt glands might also be involved in Na^+ homeostasis in *S. fruticosa* (Labidi et al. 2010), which could be a possible explanation for unchanged Na^+ content between 300 and 600 mM NaCl. Thus, growth reduction at 600 mM NaCl (despite unchanged Na^+ and effective osmotic adjustment) might be an adaptive response for reducing Na^+ build up in the tissues for long-term survival rather than salt-induced injuries. Growth reduction under salt stress might be an adaptation to increase chances of survival long enough to produce some seeds (Neumann 2011).

Exogenous application of different chemicals is reported to alleviate the harmful effects of salt stress in many crop plants (Hoque et al. 2007; Mäkelä et al. 2000; Shalata and Neumann 2001). Foliar spray with aqueous solutions of different chemicals in such studies, invokes the hypothesis that any improvement in salt tolerance by exogenous treatments could be a result of water rather than chemical itself. We therefore, tested this hypothesis and found some interesting results. Spray of distilled water increased osmolality, proline, sugars and antioxidant enzyme activities (except GPX) and reduced H_2O_2, MDA and Na^+ in 600 mM NaCl, but did not improve overall shoot growth as compared to unsprayed salt-stressed plants. Water-spray therefore appears to improve sub-cellular defense for long-term survival of plants under high salt stress. This study hints at the role of dew in arid and semi-arid coastal habitats for growth and reproduction and not just the survival of perennial halophytes growing in areas with unpredictable rainfall.

Exogenous H_2O_2 is known to improve salt tolerance of several crop plants (Gondim et al. 2010; Gong et al. 2001; Li et al. 2011; Neto et al. 2005; Wahid et al. 2007). Information about the effects of exogenous H_2O_2 on growth and salt tolerance of halophytes is not available. Although, no significant growth promotion was observed in H_2O_2-sprayed individuals of *S. fruticosa*, some parameters like ash, osmolality, proline, sugars, GSH, antioxidant enzymes activities (except GR) and Ca^{2+} were increased significantly, as in case of water-spray. Higher Ca^{2+} and reduced Na^+ in H_2O_2-sprayed plants than those of unsprayed indicate the interactive role of H_2O_2 and Ca^{2+} in Na^+ homeostasis. Wahid et al. (2007) attributed stress alleviating roles of H_2O_2 to signaling by this versatile metabolite. Exogenous H_2O_2 (0.5 mM) increased SOD and CAT activity in Oak under salt stress (Xu et al. 2008), Li et al. (2011) have reported that exogenously applied H_2O_2 (0.05 μM) decreased MDA content, enhanced GSH and increased SOD, POD, CAT and APX in salt-stressed wheat seedlings. A similar response in *S. fruticosa* indicates that sub-cellular defense mechanism is enhanced by exogenous application of H_2O_2 for long-term survival as by water spray.

Exogenous application of GB is widely reported to improve growth, survival, and tolerance of various crop plants under different stress conditions (Demiral and Türkan 2004; Díaz-Zorita et al. 2001; Harinasut et al. 1996; Rajasekaran et al. 1997). These include a decrease in leaf RWC loss in kidney bean plants under salt stress (Lopez et al. 2002), increased SOD activity in two wheat cultivars and *Prosopis ruscifolia* (Meloni and Martínez 2009; Raza et al. 2007) and reduced Na^+ and increased Ca^{2+} in wheat and eggplant (Abbas et al. 2010; Raza et al. 2007). GB treatment significantly promoted growth of our test species under high salt stress by increasing leaf osmolality, SOD activity, Ca^{2+} content and reducing MDA, H_2O_2, and Na^+. These findings indicate that GB could not only improve water uptake as an osmotic agent but also help in ion homeostasis and antioxidant defense. A small increase in proline and sugars also supports the role of GB as a probable osmotic agent.

Foliar application of AsA is also reported to enhance salt tolerance of many crops in a number of ways (Athar et al. 2008; Salama 2009; Shalata and Neumann 2001) such as by increasing soluble sugars in wheat seedlings (Al-Hakimi and Hamada 2001), decreasing lipid peroxidation in tomato (Shalata and Neumann 2001) and increasing Ca^{2+} content

in *Zea mays* and two wheat cultivars (Athar et al. 2008; Bassuony et al. 2008). Our results showed that exogenously applied AsA improved growth of *S. fruticosa* especially in 600 mM NaCl by reducing MDA and endogenous H_2O_2, increasing sugars, endogenous AsA, SOD, APX and Ca^{2+} than unsprayed controls. Our results indicate that an improved antioxidant defense and Na^+ regulation have contributed to an overall improved growth of *S. fruticosa* by AsA application.

Based on our data it may be concluded that *S. fruticosa* is an obligate halophyte that could grow optimally at 300 mM NaCl, by modulating its physiochemical performance such as H_2O_2-mediated Ca^{2+} signaling for Na^+ homeostasis and probably by enhanced OPP. Whereas at 600 mM NaCl, growth reduction appears to be an adaptive feature to reduce Na^+ build up in the organs for long-term survival (Fig. 6). Exogenous application of distilled water and H_2O_2 improved sub-cellular defense at high NaCl concentration (600 mM) although growth was unaffected. However, exogenous GB and AsA significantly improved shoot growth of *S. fruticosa* under high salt stress. This improvement in growth by GB was through improving osmotic adjustment and ion homeostasis, while exogenous AsA improved plant growth by enhancing antioxidant defense. Interestingly, biochemical changes observed in water-sprayed plants were relatively different from other exogenous treatments.

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