Acta Biologica Hungarica 61(3), pp. 299–312 (2010)
DOI: 10.1556/ABiol.61.2010.3.6

SALT EXCRETION IN *SUAEDA FRUTICOSA*

Nehla Labidi,* Manel Ammari, Dorsaf Mssedi, Maali Benzerti, Sana Snoussi and C. Abdelly

The Laboratory of Plant Adaptation to Abiotic Stress (LAPSA), Biotechnology Center at the Technopark of Borj-Cedria (CBBC), BP 901, Hammam-Lif 2050, Tunisia

(Received: June 23, 2009; accepted: September 30, 2009)

Suaeda fruticosa is a perennial "includer" halophyte devoid of glands or trichomes with a strong ability of accumulating and sequestrating Na^+ and Cl^-. We were interested in determining whether leaf cuticle salt excretion could be involved as a further mechanism in salt response of this species after long-term treatment with high salinity levels. Seedlings had been treated for three months with seawater (SW) diluted with tap water (0, 25, 50 and 75% SW). Leaf scanning electron microscopy revealed a convex adaxial side sculpture and a higher accumulation of saline crystals at the lamina margin, with a large variability on repartition and size between treatments. No salt gland or salt bladder was found. Three-dimensional wax decorations were the only structures found on leaf surface. Washing the leaf surface with water indicated that sodium and chloride predominated in excreted salts, and that potassium was poorly represented. Optimal growth of whole plant was recorded at 25% SW, correlating with maximum Na^+ and Cl^- absolute secretion rate. The leaves of plants treated with SW retained more water than those of plants treated with tap water due to lower solute potential, especially at 25% SW. Analysis of compatible solute, such as proline, total soluble carbohydrates and glycinebetaine disclosed strong relationship between glycinebetaine and osmotic potential ($r = 0.92$) suggesting that tissue hydration was partly maintained by glycinebetaine accumulation. Thus in *S. fruticosa,* increased solute accumulation associated with water retention, and steady intracellular ion homeostasis confirms the "includer" strategy of salt tolerance previously demonstrated. However, salt excretion at leaf surface also participated in conferring to this species a capacity in high salinity tolerance.

Keywords: Halophyte – includer strategy – excluder strategy – proline – glycinebetaine – salt excretion

INTRODUCTION

Although halophytes represent only 2% of terrestrial plant species, they are present in about half the higher plant families and represent a wide diversity of plant forms. Various species of *Suaeda* in the Chenopodiaceae family are used for investigating the mechanisms of ion homeostasis and water relations at cell and the whole plant level [32, 34, 45]. Among them, *Suaeda fruticosa* (L.) Frossk also presents practical

Abbreviations: DW – dry weight; EC – electric conductivity; GB – glycinebetaine; LOP – leaf osmotic potential; SEM – scanning electron microscopy; SW – seawater; TSC – total soluble carbohydrates

* Corresponding author; e-mail: hmidanehla@yahoo.com

interest, producing valuable feed for livestock in the salt marshes and salt deserts in Tunisia and exhibiting medicinal [2] and nutritive [44] properties.

To date, the few previous studies of the impact of moderate salinity on *S. fruticosa* growth led to widely divergent results, from stimulation [3, 19, 20, 41] to no effect on growth [27]. According to Mahmood et al. [27], salt tolerance of this species is linked to its ability to K^+ uptake in order to maintain higher K/Na ratios in shoots. In other studies the salt tolerance of *S. fruticosa* was mainly explained by its capacity to osmotic adjustment. This strategy was based on higher sequestration of sodium and chloride in the shoot's vacuoles [41] concomitant with its ability to synthesize osmoprotectants such as glycinebetaine (GB) in order to maintain a favorable water potential gradient and to protect cellular structures [20]. Thus, *S. fruticosa* behaves as a typical "includer" with a strong ability of accumulating and sequestrating Na^+ and Cl^- [41]. However, this conclusion was reinforced by the absence of studies mentioning salt glands or salt bladders existence's in *S. fruticosa* leaves. Compared to the excluder mechanism of *Spartina alterniflora* which is based on its capacity to control NaCl accumulation in its leaf tissues by Na^+ and Cl^- excretion, *S. fruticosa* includer mechanism has been considered as less effective, especially in high saline conditions [41]. In some halophytes such as *Odyssea paucinervis,* salt tolerance might be achieved through a combination of stress coping mechanisms including ion accumulation (includer mechanism), salt excretion (excluder mechanism) and suitable osmotic adjustment [29]. In leaves, ion exclusion may be achieved via specific secreting structures such as glands or trichomes [25, 26] or by ejection through stomatal guttation [40] and/or by cuticle diffusion at the surface leaf through an alternative way, named ''aqueous polar pores'' [38, 39].

In *S. fruticosa,* shoot Na^+ and Cl^- contents were positively correlated with the medium salinity level [41]. In plants subjected to 800 mM NaCl, leaf Na^+ content was about 10 mmol g^{-1} DW, which can be considered a high value when compared to Na^+ contents of other *Suaeda* species [7, 32]. However, the high Na^+ content of leaves may correspond simultaneously to the endocellular fraction and to the amount of salt deposited at the external leaf surface.

In this paper we further investigate the effect of salinity level on *S. fruticosa* growth, water relations, osmoticums and ion accumulation. Using scanning electron microscopy (SEM) and leaf surface washing, we analyze the importance of salt excretion.

MATERIAL AND METHODS

Culture

Cuttings of *Suaeda fruticosa* with 8 or 10 pairs of leaves were collected from the edge of Soliman "sabkha" (a salt marsh 35 km northeast of Tunis) and rooted in plastic pots filled with washed river sand (3 kg) after disinfection in saturated hypochloric calcium solution and rinse with distilled water. The experiment was carried out in a green-

house near the sea shore at Borj Cedria Biotechnology Center (CBBC), 35 km north-east of Tunis under the following conditions**:** 10 h photoperiod, mean temperatures (night-day) 19–26 °C and relative humidity (night-day) 90–60%. The cuttings were irrigated with tap water at pH 7.98, and electrical conductivity (EC) 2.8 dS m^{-1} during the first month, and then with nutrient solution [15] supplemented with Fe-K-EDTA [18] and micronutrients [16]. After two months, young plants were separated in 4 lots of 8 plants. They were irrigated with SW (pH 8, EC 50 dS m^{-1}) diluted with tap water at final concentration of 0%, 25%, 50%, or 75%. Main ions in SW include 557.0 mM Na, 10.6 mM K^+, 749.0 mM Cl^-, 3.0 mM Ca^{2+}, 1.4 mM Mg^{2+}. Main ions in tap water include 18.0 mM Na^+, 0.2 mM K^+, 16.3 mM Cl^-, 7.1 mM Ca^{2+}, 3.1 mM Mg^{2+}. Each pot was irrigated three times per week. Two harvests were made: the first one in the beginning of the treatment (initial harvest, January) and the second 3 months later. At harvest, plants were divided into leaves, stems and roots. The measured parameters were fresh and dry matter production, leaf water content, leaf osmotic potential (LOP) and Na^+, Cl^-, K^+, free proline, GB and total soluble carbohydrates (TSC) concentration in tissues. The upper leaves (3^{rd}, 4^{th}, 5^{th} and 6^{th} stages) of the oldest stem were used for the determination of secreted ions and for scanning analysis of the leaf epidermal surface. They were separated from plants and washed for 30 s with 3.5 ml of cold double-distilled water, and the washing water was sealed in air-tight vials to measure the salts secreted ions.

Growth and water relation measurements

The fresh weight was measured immediately after harvest and the dry weight (DW) after 48 h of desiccation in an oven at 60 °C. LOP was measured immediately after the plant sampling on washed leaves using the pressure chamber method [37]. The data obtained in mOsmkg were converted into MPa. Succulence index was estimated in samples according to Debez et al. [10] as the ratio of leaf fresh weight (mg) to leaf surface area (cm^{-2}) determined after scanning with Area meter (ADC Bioscientific Ltd).

Ion analysis

The leaves' washing water and samples of the grounded dry matter of the washed leaves were used for ion determination. Sodium and K^+ were assayed by flame emission spectrophotometry (Corning photometer) after nitric acid extraction (HNO_3, 0.5%). Chloride was determined on the same extract by coulometry (Haake Buchler).

Determination of organic solutes

For organic solute analysis, four replicates from fresh matter (ca. 100 mg samples) of the upper leaves were used. Proline was extracted and estimated as in [1], using a

calibration curve prepared with pure proline. Proline content was expressed as μmol g^{-1} DW after correction for the humidity in the leaves.

For TSC determination, fresh material was exhaustively extracted in boiling 80% (v/v) ethanol. Ethanol-soluble extracts were dried in a Turbovap LV evaporator (Zymark Corp, Hopkinton, MA, USA) and soluble compounds were redissolved with 4 ml of distilled water, mixed and centrifuged at 20,000 × *g* for 10 min. Total soluble carbohydrates were determined by the anthrone method [42]. Leaf (100 μl) extract was added to 3 ml (final volume) assay medium containing 1.08 M H_2SO_4, 1.09 mM thiourea and 2.1 mM anthrone. The mixture was heated at 100 °C for 10 min and absorbance was read at 620 nm. A calibration curve with D-glucose was used as a standard. TSC was expressed as μmol g^{-1} DW after correction for leaf water content.

Quaternary ammonium compounds were extracted and measured as GB equivalents according to Grieve and Grattan [13]. Dried and finely grounded lyophilized leaf samples were mechanically shaken with 20 ml of deionized H_2O for 24 h at 25 °C. The samples were then filtered and the filtrates were diluted (1 : 1) with 2 N H_2SO_4. Aliquots (0.5 ml) were taken into centrifuge tubes and cooled in ice water for 1 h. Cold KI-I_2 reagent (0.20 ml) was added and then reactants were gently stirred (KI-I_2 reagent: 17.5 g of iodine and 20 g of potassium iodide were dissolved in 100 ml of distilled water). The tubes were stored at 4 °C for 16 h and then centrifuged at 10,000 rpm for 15 min at 0 °C. The supernatant was carefully aspired with a fine tipped glass tube. The periodide crystals were dissolved in 9.0 ml of 1.2-dichloroethane and mixed vigorously. After 2 h, the absorbance was measured at 365 nm. Reference standards of GB were prepared in 1 N H_2SO_4.

Scanning electron microscopy

The 3rd or 4th pair of leaves from old stems was observed and photographed with SEM (FEI quanta 200). All leaves were carefully washed before the beginning of treatments. Care was taken during culture and irrigation to avoid accidental salt projection and deposition. The epidermal surface was examined directly on three micrographs randomly taken of the middle portion or at the margin of different fresh leaves. Each micrograph had known dimensions, and so a defined surface area which corresponded to a determined leaf surface area. Micrographs taken in a repeat of the experiment one year later gave similar results.

Statistical analysis

All data presented are mean values of 4 replicates for GB, proline and TSC, and 6 replicates for biomass, ion contents and ion secretion. Data were subjected to a one-way analysis of variance (ANOVA), using SAS™ software (SAS Institute Inc., 1989).

RESULTS

Growth

The highest dry matter production was produced by plants irrigated with 25% SW (Fig. 1). Leaf and stem biomass production of these plants were 2- and 1.5-fold larger than those of control plants (irrigated with tap water), respectively. Furthermore, it is clear from Fig. 1 that 25% SW treatment stimulated dry matter allocation to the shoot, since root growth was the same at 0, 25 and 50% SW.

Dry matter of plants treated with 50 and 75% SW was similar to that of plants irrigated by tap water (0% SW concentration). Thus, *S. fruticosa* behaves as an obligate halophyte.

Scanning electron microscopy examination of the leaf surface

The upper leaves from old stems were observed and photographed with SEM. The epidermal leaves of all plants, irrespectively of the treatment, showed salt excretion recognizable through the presence of cubic crystal deposits on all leaf surfaces (Figs 2, 3). These crystals had different sizes and were constituted with a single cubic submicrometer-sized particle or with an aggregate of smaller particles or with large crystals (Fig. 2). The larger crystals were located at the margins. Smaller crystal particles were detected in more central regions on the adaxial and the abaxial sides (Fig. 2). Even at higher resolution (Fig. 3), no specific salt secretion structure such as gland and bladder appeared. However, the outer layer of the leaf epidermis cells was densely covered with wax.

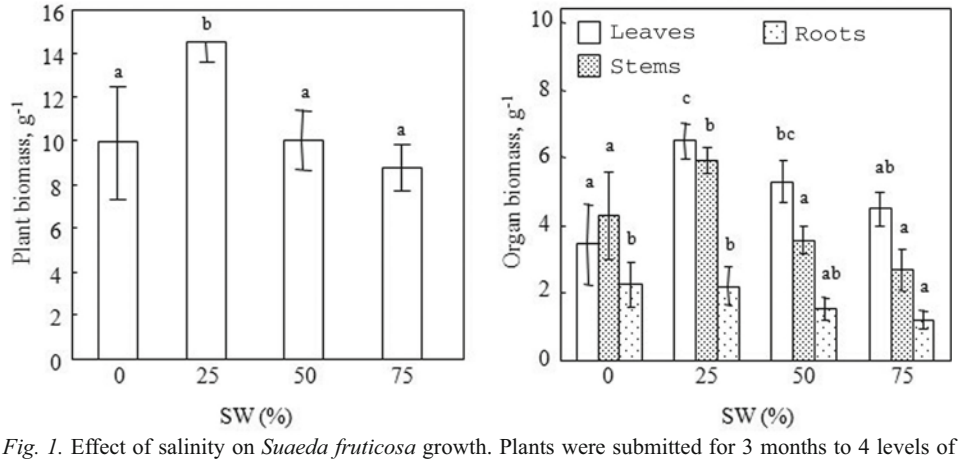

Fig. 1. Effect of salinity on *Suaeda fruticosa* growth. Plants were submitted for 3 months to 4 levels of salinity (irrigation with 0, 25, 50 and 75% seawater). Values are means ± SE of 6 measurements. For each organ, values followed by the same letter are not significantly different (p = 0.05) as predicted by Duncan's test

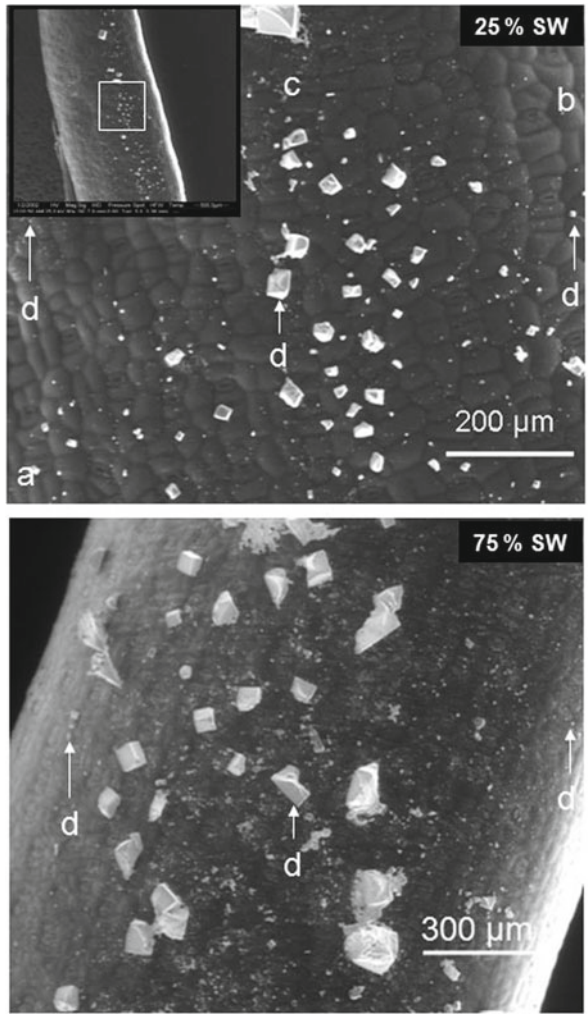

Fig. 2. Scanning electron micrographs showing salt excretion at leaf surface. *S. fruticosa* plants were submitted for 3 months to 2 levels of salinity (irrigation with 25 and 75% seawater). Note that small cubic saline crystals (d) with different sizes were detected on adaxial (a) and on abaxial (b) side and larger crystals (d) were located at the margin (c)

Ion accumulation, K/Na rate and secretion in washed leaves

Some upper leaves of the old stems were harvested and washed. The ion content of the leaves (Fig. 4) and the washings was then determined (Table 1). In leaves of salt-treated plants, Na^+ and Cl^- were the main ions. The internal Na^+ content of washed leaves, which exceeded that of Cl^-, increased in response to 25 and 50% SW irriga-

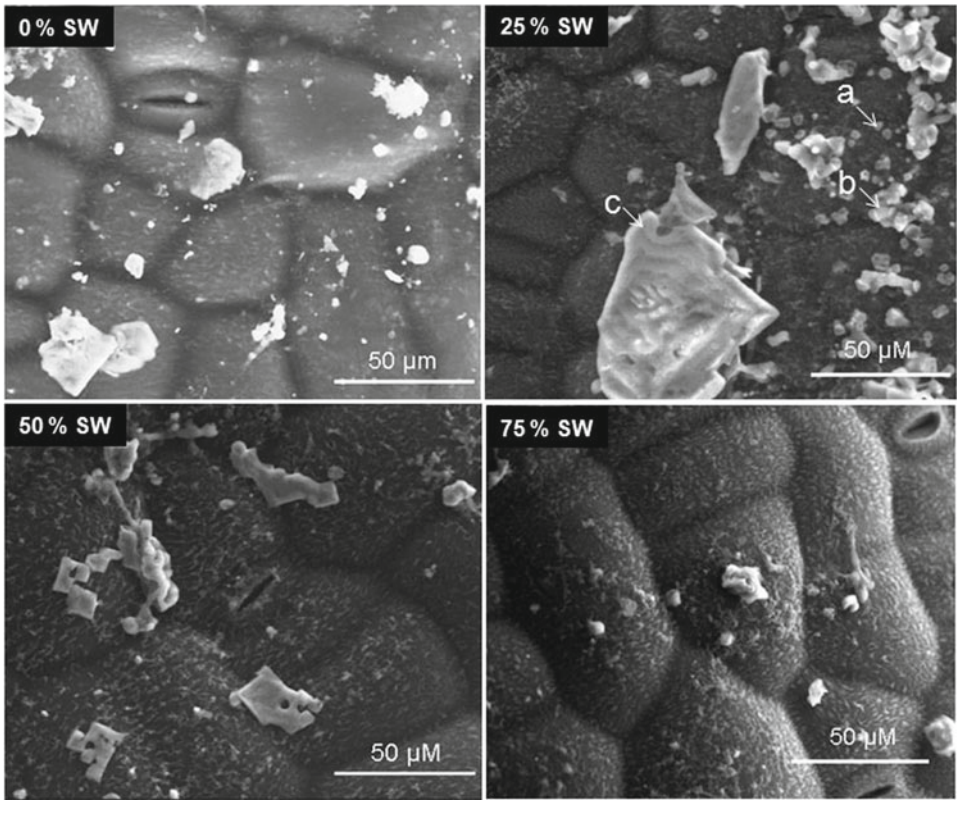

Fig. 3. Scanning electron micrographs showing leaf surface structure and salt excretion at the leaf margins. *S. fruticosa* plants were submitted for 3 months to 4 levels of salinity (irrigation with 0, 25, 50 and 75% seawater). Note that cubic saline crystals were detected in all treatments (a: single cubic submicrometer-sized particle, b: aggregate of smaller particles, c: large cluster) and that wax deposition and surface rugosity increased with salinity

tion. However, no statistically significant difference was observed between control and 50% SW. The internal leaf Cl^- content increased similarly in all SW treatments. However, the internal leaf K^+ content (Fig. 5) markedly increased in 25% SW but was not significantly changed in 50 and 75% SW in comparison to the control. The K^+/Na^+ ratio (Fig. 4) decreased slightly at 50% SW, due to stable K^+ content accompanied with increased Na^+ accumulation in leaves.

Neither Na^+ nor K^+ concentrations in washed leaves did depend on treatment (Fig. 4): Na^+ concentration remained in the 5.0–5.5 mmol g^{-1} DW range, and K^+ concentration in the 0.5–0.6 mmol g^{-1} DW range. On the contrary, Cl^- concentration increased with medium salinity, from 1.61 mmol g^{-1} DW (0% SW) to 4.31 mmol g^{-1} DW (75% SW).

In order to assess the efficiency of the excretion process at controlling the internal ion concentrations, we compared the absolute excretion and the relative excretion of

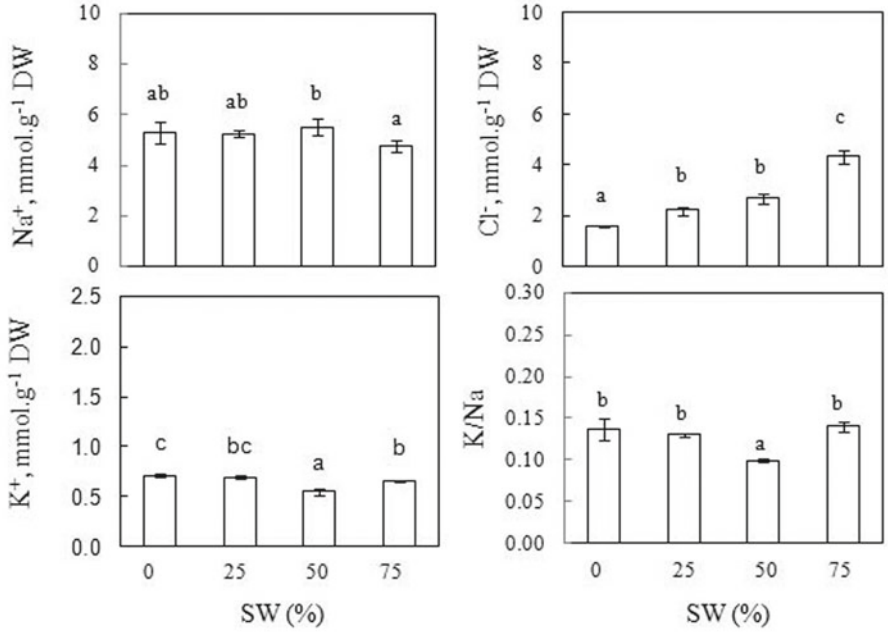

Fig. 4. Effect of salinity on ion concentration and K/Na ratio in leaves. *S. fruticosa* plants were submitted for 3 months to 4 levels of salinity (irrigation with 0, 25, 50 and 75% seawater). Leaves (3rd, 4th, 5th and 6th stage) of old stems were washed for 30 s to eliminate the salt secreted at their surface. Values are means ± SE of 6 measurements. Values followed by the same letter are not significantly different ($p < 0.05$) as predicted by Duncan's test

Table 1

Absolute excretion rate (AER(1)) and relative excretion rate (RER(2)) of Na^+, Cl^- and K^+ at the leaf surface of *S. fruticosa* irrigated for 3 months with 4 saline levels (0%, 25%, 50% and 75% SW concentration)

Ion	Parameter	Seawater (%)			
		0	25	50	75
Na^+	AER	0.229±0.026 a	0.366±0.045 b	0.239±0.019 a	0.325±0.045 b
Na^+	RER	32% a	33% a	31% a	42% b
Cl^-	AER	0.047±0.006 a	0.217±0.006 c	0.161±0.007 b	0.312±0.042 d
Cl^-	RER	30% a	70% b	60% b	76% b
K^+	AER	0.041±0.005 a	0.134±0.042 b	0.028±0.003 a	0.066±0.011 a
K^+	RER	38% ab	54% c	34% a	51% bc

Values are means ± SE of 6 measurements. For each line, values followed by the same letter are not significantly different ($p = 0.05$) as predicted by Duncan's test.

(1)AER – absolute excretion rate (μmol cm^{-2} d^{-1}), is estimated as (ion contents of leaf washing / leaf area of the leaf washed / treatment period).

(2)RER – relative excretion rate (%), is estimated as 100× (ion content of leaf washing water / (ion content of leaf washing water + ion contents of the washed leaf)

ions. The rate of the absolute excretion of ions was expressed as the concentration of ion in the leaf washing water per unit of leaf surface area washed and unit of time (μmol cm^2 d^{-1}). Results (Table 1) showed that Na^+ and Cl^- were the major ions secreted from the leaves. Higher absolute excretion rates of Na^+ and Cl^- were found at 25% and at 75% SW. Absolute excretion rate was lower for Cl^- than for Na^+ secreted rate between 0 and 50% SW, and reached approximately the level of Na^+ absolute excretion rate at 75% SW. In all treatments, the absolute K^+ secretion rate was much lower than those of Na^+ and Cl^-.

The relative excretion rate was estimated as the amount of salt excreted as a proportion of that allocated to the fresh weight of leaves over 90 days of treatment [excreted salt / (excreted salt + accumulated salt)]. The proportion of the Na^+ excreted by the leaves represented 33% of that allocated at 0, 25 and 50% SW and it increased to 42% at 75% SW. Higher relative rates of Cl^- were observed at all salinity levels, with values exceeding 70%. Surprisingly, high values of the relative rate of K^+ excretion were found (more than 50% of allocated K^+ at 25 and 75% SW). These results suggested that the excretion mechanism did not distinguish between Na^+ and K^+.

Leaf water relations

Salinity significantly modified tissue water content assessed by leaf succulence (Fig. 5). This parameter (mg cm^{-2}) increased at all SW levels, and showed its highest values at 25% SW. The LOP of *S. fruticosa* of washed leaves was generally higher in control plants, and decreased at all SW levels. Reduction of osmotic potential was marked in plants irrigated with 25% SW but was not significant at 75% SW when compared to the control.

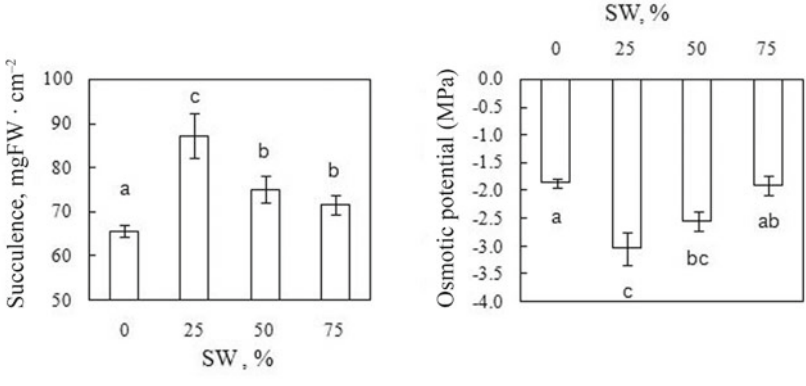

Fig. 5. Effect of salinity level on leaf water relationships. *S. fruticosa* plants were submitted for 3 months to 4 levels of salinity (irrigation with 0, 25, 50 and 75% seawater). Leaf osmotic potential was determined on washed leaves

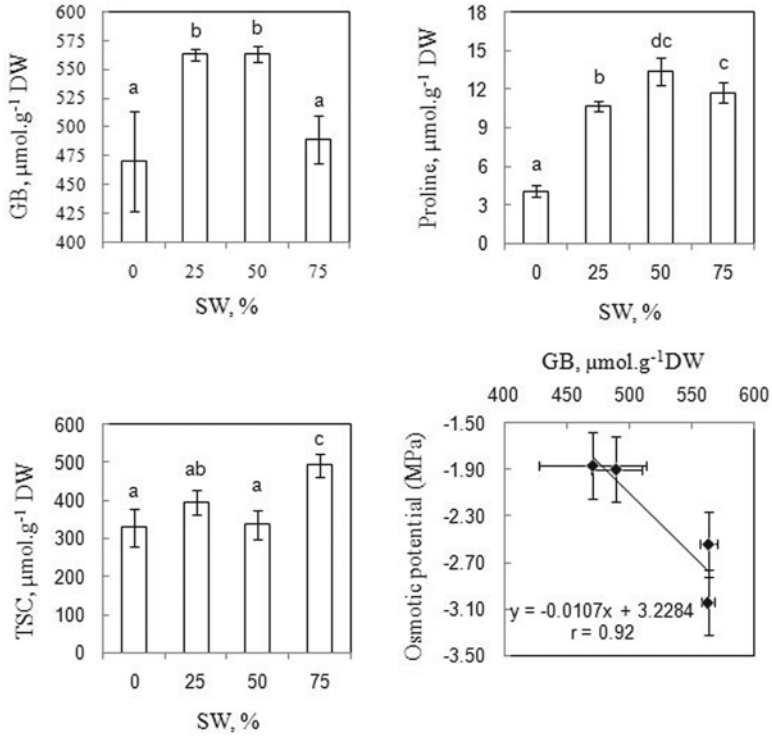

Fig. 6. Effect of salinity on glycinebetaine (GB), proline and total soluble carbohydrates (TSC) in leaves. *S. fruticosa* plants were submitted for 3 months to 4 levels of salinity (irrigation with 0, 25, 50 and 75% seawater). The right lower panel shows correlation of data of left upper panel with those of Fig. 5

Organic solutes

In plants treated with SW, proline concentrations in leaves significantly increased with salinity level and reached high levels at 50 and 75% SW (Fig. 6). Similarly, GB concentration showed large increase at 25 and 50% SW. Linear regression analysis indicated a strong correlation between GB and osmotic potential ($r = 0.92$). Finally, TSC increased only at 75% SW.

DISCUSSION

Maximal growth stimulation, as compared to control without added salt, occurred at 25% SW evidencing the facultative halophytism of *S. fruticosa*. Growth was just maintained at control value at higher salinity levels, suggesting that some detrimental effect of excess salt balanced the above-stimulating effect. Previous studies demonstrated that the mechanism of salt tolerance in *S. fruticosa* could involve a delicate

balance between growth rate and ion accumulation rate, involving production of osmotic compounds, osmotic adjustment, and maintenance of pressure potential as well as high K/Na ratio in shoots [20, 27]. The aim of the present study was to find further traits for salt tolerance in this species. Our results provide two lines of evidence for the involvement of salt excretion as an efficient additional mechanism for salinity tolerance in *S. fruticosa*: (i) salt crystals mainly composed of NaCl were present at leaf surfaces, at all salinity levels; (ii) washing the leaves eliminated significant amounts of Na^+ , diminishing leaf Na^+ content by ca. 38%.

Thus, *S. fruticosa* leaves accumulated and preferentially secreted Na^+ and then $Cl^–$; consequently lowering the ratio of harmful-to-nutrient ions (e.g. Na^+/K^+) to a level compatible with plant survival. This result is in agreement with other studies which reported that Na^+ and $Cl^–$ were the major ions secreted from the leaves of halophyte species [14, 29]. Comparison of salt secretion rates among studies is difficult due to differences in environmental conditions and plant factors. However, it is interesting to note that the highest rate of secretion for *S. fruticosa* (366 nmol Na^+ cm^{-2} d^{-1} at 25% SW) was similar to that reported for gland salt secretion of *Spartina anglica* (383 nmol Na^+ cm^{-2} d^{-1}) at a 0.1 M NaCl [36]. Thus it seems that salt secretion is an important mechanism of salt control in *S. fruticosa*. We speculate that the rapid rates of secretion above 25% SW (per surface leaf area unit, Table 1) is determinant for the tolerance to salinity of this species. In *Atriplex marina* an increasing salt excretion rate over the range of 0 to 100% SW maintains a constant leaf salt content [11]. In this species, maximal growth was observed at 50% SW [8]. In our investigation, maximal growth activity at 25% was correlated with maximal K^+, Na^+ and $Cl^–$ absolute secretion rates.

Leaves are thought to have limited ability to store toxic ions in outside key metabolic sites, so the excess ions must be ejected by stomatal guttation [40] and/or by cuticle diffusion at the surface leaf. Recently, it has been shown that lipoidic compounds, such as waxes, diffuse through the cuticle via a lipoidic pathway [5, 35], whereas ions diffuse through an alternative way, called ‘‘aqueous polar pores’’ [38, 39], composed of the polar groups of the cutin network [6]. Modelling and calculation of the molecular structure of the cuticular matrix revealed an average pore radius between 0.3 and 0.5 nm [31, 38] not visualized until now [24]. In our study, we have observed convex sculptures at leaf adaxial surface and a higher accumulation of saline crystals at the leaf margins. The convex structures were densely covered with three-dimensional waxes which are water repellent [24]. Thus, salt crystals particles may be carried to the leaf margin by water droplets along the waxy convex structures. This self-cleaning process results in a smart protection against particle accumulation [30] and maintains CO_2 accessibility for photosynthesis [4]. The movement of water droplets on the leaf surface is complex. It depends on the surface roughness, on the leaf surface contact angle and on wettability [24]. This complexity could be responsible for the larger variability on the saline crystal’s repartition and size between treatments.

Finally, the high and constant Na^+ content (5 mmol g^{-1} DW) within the leaf tissues (washed leaves) suggest that *S. fruticosa* could be used for bioremediation of salt-

affected soils. Indeed, Rabhi et al. [33] found a reduction of the soil-soluble Na^+ content inside the tufts of *S. fruticosa* as compared with the soil outside tufts in the natural biotope of this species during the driest period of the year.

The Na^+ hyperaccumulation inside leaf cells (as observed in washed leaves) and the absence of growth disturbance in all treatments strongly suggest that the cytoplasmic metabolic machinery is protected against excess ion concentration. Presumably, intracellular salt was sequestrated outside the cytoplasm, in the cell vacuoles. Vacuolar H^+-ATPase generates the proton driving force which activates secondary ion transport toward the vacuole in other halophytes [9, 17]. Vacuolar compartmentation is an essential mechanism for salt-tolerance, since it lowers cytosolic Na^+ levels while contributing to osmotic adjustment for cell turgor and expansion [43]. Measurements of plant water relations indicated that *S. fruticosa* plants adjusted their LOP to more negative levels at 25 and 50% SW treatment. However, the stability of Na^+ concentration among all SW treatments in washed leaves suggested that Na^+ was not responsible for osmotic adjustment at 25 or 50% SW. At these SW concentrations, we have observed an increase in the leaf succulence. Leaf succulence enables the dilution of internal ion content [9] and lowers the metabolic cost for the production of osmolytes [32]. The correlative response of leaf GB content to the change of osmotic potential ($r = 0{,}92$) supports the previous conclusion of Khan et al. [20] which suppose that GB can be accumulated in *S. fruticosa* in response to saline stress as an organic osmolyte. Yet, the osmotic potential in the cytoplasm can be also controlled by K^+ [21]. In the present study, K^+ contents decreased only at 50% SW. Thus long-term osmotic response seems not to be associated with inorganic osmoticums such as K^+. However, the relative importance of inorganic versus organic osmoticums in osmotic homeostasis is likely tissue specific. For example, highly vacuolated mesophyll cells may heavily rely on inorganic ions (Na^+ and K^+) for osmotic adjustment, whereas epidermal cells with proportionally smaller vacuoles may rely more on organic molecules and K^+ [46]. At high salinity (75% SW), the whole plant growth reduction was not caused by a reduced ability to adjust osmotically in leaves, but probably by other factors [12] such as the high accumulation of Na^+ and Cl^- in the stems.

In conclusion, our investigation discloses that salt excretion may be involved as an efficient additional mechanism of salt tolerance in *S. fruticosa.* No specific structures have been found on the leaf surface cuticle. Nevertheless, the secretion rates of Na^+ and Cl^- which may be caused by stomatal guttation and/or by cuticle diffusion was similar to that reported for gland salt. The convex waxy sculptures of the leaf adaxial surface may protect the major photosynthesizing leaf side against the accumulation of saline crystals which are moved to the leaf margins.

REFERENCES

1. Bates, L. S., Waldren, R. P., Teare, I. D. (1973) Rapid determination of free proline for water stress studies. *Plant Soil 39*, 205–207.
2. Benwahhoud, M., Jouad, H., Eddouks, M., Lyoussi, B. (2001) Hypoglycemic effect of *Suaeda fruticosa* in streptozotocin induced diabetic rats. *J. Ethnopharm. 76*, 35–38.

3. Boucaud, J., Ungar, I. A. (1978) Halophile et resistance au sel dans le genre *Suaeda* Forssk. *Bulletin de la Société Botanique de France*, *Actualité Botanique 34*, 23–35.
4. Brewer, C. A., Smith, W. K., Vogelmann, T. C. (1991) Functional interaction between leaf trichomes, leaf wettability and the optical properties of water droplets. *Plant Cell Environ. 14*, 955–962.
5. Buchholz, A., Schönherr, J. (2000) Thermodynamic analysis of diffusion of non electrolytes across plant cuticles in the presence and absence of the plasticiser tributyl phosphate. *Planta 212*, 103–111.
6. Chamel, A., Pineri, M., Escoubes, M. (1991) Quantitative determination of water sorption by plant cuticles. *Plant Cell Environ*. *14*, 87–95.
7. Clipson, N. J. W., Flowers, T. J. *(*1987) Salt tolerance in the halophyte *suaeda maritima* (l.) dum. The effect of salinity on the concentration of sodium in the xylem. *New Phytol*. *105*, 359–366.
8. Connor, D. J. (1969) Growth of the grey mangrove *Avicennia marina* in nutrient culture. *Biotropica 1*, 36–40.
9. Debez, A., Koyro, H. W., Grignon, C., Abdelly, C., Huchzermeyer, B. (2008) Relationship between the photosynthetic activity and the performance of *Cakile maritima* after long-term salt treatment. *Physiol. Plant. 133*, 373–385.
10. Debez, A., Saadaoui, D., Ramani, B., Ouerghi, Z., Koyro, H. W., Huchzermeyer, B., Abdelly, C. (2006) Leaf H^+-ATPase activity and photosynthetic capacity of *Cakile maritima* under increasing salinity. *Environ. Exp. Bot. 57,* 285–295.
11. Drennan, P., Pammenter, N. W. (1982) Physiology of salt secretion in the mangrove *Avicennia marina* (Forsk.) Vierh. *New Phytol*. *91*, 597–606.
12. Gale, J., Zeroni, M. (1984) Cultivation of plants in brackish water incontrolled environmental agriculture. In: Staples, R. C., Toenniessen, G. R. (eds) *Salinity Tolerance in Plants.* John Wiley and Sons, New York, 363–380.
13. Grieve, C. M., Grattan, S. R. (1983) Rapid assay for determination of water-soluble quaternary ammonium-compounds. *Plant Soil 70*, 303–307.
14. Hameed, M., Ashraf, M. (2008) Physiological and biochemical adaptations of *Cynodon dactylon* (L.) Pers. from the Salt Range (Pakistan) to salinity stress. *Flora 203*, 683–694.
15. Hewitt, E. J. (1966) *Sand and water culture methods used in the study of plant nutrition.* Commonw. Agr. Bur. Tech. Commun. 22. (revised 2nd ed.).
16. Hoagland, D. R., Arnon, D. R. (1950) Growing plants without soil by the water-culture method. *Calif. Agric. Exp. Sta. Cir. 347,* 1–32.
17. Hong-Bo, S., Li-Ye, C., Ming-An, S., Shi-Qing, L., Ji-Cheng, Y. (2008) Bioengineering plant resistance to abiotic stresses by the global calcium signal system. *Biotechnol. Adv. 26*, 503–510.
18. Jacobson, L. (1951) Maintenance of iron supply in nutrient solutions by a single addition of ferric-potassium-ethylene-diamine-tetraacetate. *Plant Physiol. 26*, 411–413.
19. Kefu, Z., Hai, F., Harris, P. J. C. (1995) The physiological basis of growth inhibition of halophytes by potassium. In: Khan, M. A., Ungar, I. A. (eds) *Biology of Salt Tolerant Plants.* Department of Botany, University of Karachi, Karachi, pp. 221–227.
20. Khan, M. A., Ungar, I. A., Showalter, A. M. (2000) The effect of salinity on the growth, water status, and ion content of a leaf succulent perennial halophyte, *Suaeda fruticosa* (L.) Forssk. *J. Arid Environ. 45*, 73–84.
21. Kirst, G. O. (1989) Salinity tolerance of eukaryotic marine algae. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. *40*, 21–53.
22. Kleber, H. P., Schlee, D., Schöpp, W. (1987) *Biochemisches Praktikum.* 2. Aufl. G. Fischer, Jena.
23. Koch, K., Bhushan, B., Barthlott, W. (2009) Multifunctional surface structures of plants: An inspiration for biomimetics. *Prog. Mater. Sci. 54*, 137–178.
24. Koch, K., Ensikat, H. J. (2008) The hydrophobic coatings of plant surfaces: epicuticular wax crystals and their morphologies, crystallinity and molecular self-assembly. *Micron 39*, 759–772.
25. Koyro, H.-W., Geissler, N., Hussin, S. (2009) Survival at Extreme Locations: Life Strategies of Halophytes. *Salinity and Water Stress 44*, 167–177.
26. Lefèvre, I., Marchal, G., Meerts, P., Corréal, E., Lutts, S. (2009) Chloride salinity reduces cadmium accumulation by the Mediterranean halophyte species *Atriplex halimus* L. *Environ. Exp. Bot. 65*, 142–152.

27. Mahmood, K., Malik, K. A., Lodhi, M. A. K., Sheikh, K. H. (1996) Seed germination and salinity tolerance in plant species growing on saline wetlands. *Biol. Plant. 38*, 309–315.
28. Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S.-I., Lee, Y. C. (2005) Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. *Anal. Biochem. 339*, 69–72.
29. Naidoo, G., Somaru, R., Achar, P. (2008) Morphological and physiological responses of the halophyte, *Odyssea paucinervis* (Staph) (Poaceae), to salinity. *Flora 203*, 437–447.
30. Neinhuis, C., Barthlott, W. (1997) Characterization and distribution of water-repellent, self-cleaning plant surfaces. *Ann. Bot. 79*, 667–677.
31. Popp, C., Burghardt, M., Friedmann, A., Riederer, M. (2005) Characterization of hydrophilic and lipophilic pathways of *Hedera helix* L. cuticular membranes: permeation of water and uncharged organic compounds. *J. Exp. Bot*. *56*, 2797–2806.
32. Qiu, N., Chen, M., Guo, J., Bao, H., Ma, X., Wang, B. (2007) Coordinate up-regulation of V-H^+-ATPase and vacuolar Na^+/H^+ antiporter as a response to NaCl treatment in a C3 halophyte *Suaeda salsa. Plant Sci. 172*, 1218–1225.
33. Rabhi, M., Hafsi, C., Lakhdar, A., Hajji, S., Barhoumi, Z., Hamrouni, M. H., Abdelly, C., Smaoui, A. (2009) Evaluation of the capacity of three halophytes to desalinize their rhizosphere as grown on saline soils under nonleaching conditions. *Afr. J. Ecol. 47,* 463–468.
34. Ravindran, K. C., Venkatesan, K., Balakrishnan, V., Chellappan, K. P., Balasubramanian, T. (2007) Restoration of saline land by halophytes for Indian soils. *Soil Biol. Biochem. 39*, 2661–2664.
35. Riederer, M., Schreiber, L. (1995) Waxes-the transport barriers of plant cuticles. In: Hamilton, R. J. (ed.) *Waxes: Chemistry, Molecular Biology and Functions*. The Oily Press, Dundee, Scotland, pp. 131–156.
36. Rozema, J., Gude, H., Pollak, G. (1981) An ecophysiological study of the salt secretion of four halophytes. *New Phytol*. *89*, 201–217.
37. Scholander, P. F., Hammel, H. T., Bradstreet, E. D., Hemmingsen, E. A. (1965) Sap pressure in vascular plants. *Science 148*, 339–346.
38. Schönherr, J. (2002) A mechanistic analysis of penetration of glyphosate salts across astomatous cuticular membranes. *Pest Manage. Sci*. *58*, 343–351.
39. Schönherr, J. (2006) Characterization of aqueous pores in plant cuticles and permeation of ionic solutes. *J. Exp. Bot*. *57*, 2471–2491.
40. Shi-qing, L. I., Chun-rong, J. I., Ya-ning, F., Xiao-li, C., Sheng-xiu, L. I. (2008) Advances in nitrogen loss leached by precipitation from plant canopy. *Agric. Sci. China 7*, 480–486.
41. Sleimi, N., Abdelly, C. (2003) Salt tolerance strategy of two fodder halophytes species: *Spartina alterniflora* and *Suaeda fruticosa.* In: Lieth, H., Mochtchenko, M. (eds) *Cash Crop Halophytes:* Recent Studies. Kluwer Academic Publishers, pp. 79–86.
42. Spiro, R. G. (1966) Characterization of carbohydrate units of glycoproteins. In: Colowick, S. P., Kaplan, N. O. (eds) *Methods in Enzymology 8.* London & New York: Academic Press, pp. 26–52.
43. Szepesi, A., Csiszár, J., Gémes, K., Horváth, E., Horváth, F., Simon, M. L., Tari, I. (2009) Salicylic acid improves acclimation to salt stress by stimulating abscisic aldehyde oxidase activity and abscisic acid accumulation, and increases Na^+ content in leaves without toxicity symptoms in *Solanum lycopersicum* L. *J. Plant Physiol. 51*, 145–150.
44. Weber, D. J., Ansari, R., Gul, B., Khan, M. A. (2007) Potential of halophytes as source of edible oil. *J. Arid Environ. 68*, 315–321.
45. Yang, C., Shi, D., Wang, D. (2008) Comparative effects of salt and alkali stresses on growth, osmotic adjustment and ionic balance of an alkali-resistant halophyte *Suaeda glauca* (Bge). *Plant Growth Regul*. *56*, 179–190.
46. Ye, C. J., Zhao, K. F. (2003) Osmotically active compounds and their localization in the marine halophyte eelgrass. *Biol. Plant 46*, 137–140.