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Salt tolerance in the halophyte *Salicornia dolichostachya* Moss: growth, morphology and physiology

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Abstract

Salinization of agricultural land is an increasing problem. Because of their high tolerance to salinity, *Salicornia* spp. could become models to study salt tolerance; they also represent promising saline crops. The salinity-growth response curve for *Salicornia dolichostachya* Moss was evaluated at 12 salt concentrations in a hydroponic study in a greenhouse and at 5 different seawater dilutions in an outside setting. Salt concentrations ranged between 0 mM and 500 mM NaCl (≈seawater salinity). Plants were grown for six weeks and morphological and physiological adaptations in different tissues were evaluated.

S. dolichostachya had its growth optimum at 300 mM NaCl in the root medium, independent of the basis on which growth was expressed. The relative growth rate (RGR) in the greenhouse experiment was comparable with RGR-values in the outdoor growth experiment.

Leaf succulence and stem diameter had the highest values at the growth optimum (300 mM NaCl). Carbon isotope discrimination ($\delta^{13}C$) decreased upon salinity. *S. dolichostachya* maintained a lower leaf sap osmotic potential relative to the external solution over the entire salinity range, this was mainly accomplished by accumulation of Na^+ and Cl^-. Glycine betaine concentrations did not significantly differ between the treatments. Na^+:K^+-ratio and K^+-selectivity in the shoots increased with increasing salinity, both showed variation between expanding and expanded shoot tissue. We conclude that *S. dolichostachya* was highly salt tolerant and showed salt requirement for optimal growth. Future growth experiments should be done under standardized conditions and more work at the tissue and cellular level needs to be done to identify the underlying mechanisms of salt tolerance.

Keywords: *Salicornia dolichostachya* Moss, salinity tolerance, salt stimulated growth, Na^+:K^+-ratio and K^+-selectivity, glycine betaine

1. Introduction

The need for salt tolerant crops increases as substantial percentages of cultivated land worldwide are affected by salinity (FAO 2002). The prospect is that fresh water will become scarce as a result of an increasing demand of the growing world population (UN 2010). Moreover, as a result of global warming, arable land may suffer from increasing saline and dry conditions in the future, whereas sea level rise will particularly threaten coastal lowlands. Most conventional crop species are salt sensitive, e.g. rice, chickpea, corn and most fruit crops. Plants growing on saline soils face several challenges. Water uptake is hampered because of the low water potential of the soil (Munns and Tester, 2008). Salts accumulate within the plant to toxic

levels (Greenway and Munns, 1980) and deficiencies of essential nutrients occur, e.g. K^+ (Flowers and Colmer, 2008; Flowers et al., 1986). Plants that naturally possess the traits needed to grow and reproduce on saline soils at salt concentrations of at least 200 mM are termed halophytes by Flowers and Colmer (2008). By studying the diverse set of mechanisms that halophytes employ to deal with salt, we might gain insight into which parameters are most promising to target for increasing salt tolerance in conventional crops. The taxonomic diversity of halophytes prompted Glenn et al. (1999) to predict the prospective of introducing salt tolerance in crops by small changes in the genetic makeup, although this has proved to be fairly difficult thus far (Flowers and Flowers, 2005). Alternatively, domestication of halophytes can convert them into high yielding crops (Rozema and Flowers, 2008) or halophytes can be used for remediation and reclamation of salt affected lands (Barrett-Lennard, 2002).

The plant order of the Caryophyllales contains the highest percentage of halophytic species amongst all orders of flowering plants (Flowers et al., 2010), 21.4% of all halophytic species are within this order. Within this order the family of the Amaranthaceae comprises the highest number of halophytic genera (Flowers et al., 1986). The genus *Salicornia* belongs to the Amaranthaceae and consists of highly salt tolerant annuals without salt glands or salt bladders. *Salicornia* spp. occur in saline environments around the globe, except for Australia and South America where other salt tolerant members of the Salicornioideae occur (Kadereit et al., 2007). There is no general consensus of the number of *Salicornia* species due to the large phenotypic variation between the same species and morphological parallelism between different species (Davy et al., 2001; Kadereit et al., 2007). The estimations lie around 25–30 species in the genus (Kadereit et al., 2007). Several economically viable applications have been suggested for *Salicornia* species. *Salicornia bigelovii* has been proposed as a promising oil seed crop with

yields comparable to conventional crops under non-saline conditions (Glenn et al., 1991). *Salicornia* spp. have been successfully grown in aquaculture systems (Grattan et al., 2008) and can be used for human food consumption. Due to the high salt tolerance, high growth rate, short generation time, its capability of producing many seeds and agronomic value, *Salicornia* spp. can become valuable model species to study salt tolerance mechanisms and as a saline crop.

Salicornia dolichostachya Moss is a tetraploid species that is native to The Netherlands below the mean high water line (MHWL) and is subjected to flooding on a regular basis (Rozema 1987). The present study determined the salinity-growth response curve for *S. dolichostachya* in hydroponic culture at 10 external NaCl concentrations with small intervals between salt concentrations (50 mM NaCl). Additionally, the growth response curve was determined in two seawater treatments in the greenhouse and compared with the growth response of plants grown in an outside setting. We tried to characterize the morphological and physiological traits underlying the growth response. Succulence, stem diameter, $\delta^{13}C$, osmotic potential of leaf sap and tissue solute concentrations (Na^+, Cl^-, K^+, Mg^{2+} and glycine betaine) were measured in root tissue and expanding - and expanded succulent shoot tissue.

2. Materials and methods

2.1 Plant material

Seeds of *Salicornia dolichostachya* were collected below the mean high water line (MHWL) at Lutjesstrand, Wieringen, The Netherlands in November 2010. Before sowing, seeds were stored dry in a refrigerator at 7 ºC for 50 d, to simulate a stratification period. Two separate experiments were done. In experiment 1 we investigated the effect of a range of NaCl concentrations in the root medium (0-5-50-100-150-200-250-300-400-500 mM NaCl) and two

111 seawater treatments (electric conductivity (EC) 20 and 40 dS/m, this is ~210 and ~420 mM
112 NaCl, respectively) on growth and a set of physiological parameters in *S. dolichostachya* and this
113 experiment was conducted in a greenhouse. In experiment 2 we grew *S. dolichostachya* in an
114 inland seawater drip-irrigation system, to be able to compare the relative growth rate (RGR) in
115 experiment 1 to the RGR of the same species in an outside setting.
116
117 *2.2 Experiment 1: Greenhouse growth experiment*
118 *Plant growth and NaCl treatments.* Seeds were sown on peat soil (seed pot soil;
119 Jongkind, Aalsmeer, The Netherlands) and grown for 61 d. Seedlings were washed free of soil
120 before being transplanted individually in black 1-L polyethylene pots containing (in mM): K^+,
121 3.001 ; Ca^{2+}, 2 ; Mg^{2+}, 0.5 ; NO_3^-, 5 ; NH_4^+, 1.001 ; HPO_4^{2-}, 1 ; SO_4, 0.516 ; Cl^-, 0.001 ; $H_2BO_3^-$,
122 0.025 ; Mn^{2+}, 0.002 ; Zn^{2+}, 0.002 ; Cu^{2+}, 0.001 ; Mo^{2+}, 0.001 ; Fe-Na-EDTA, 0.01, buffered with
123 2 mM MES, (pH 6.0). Seedlings were grown for 14 d to recover from transplanting; over these
124 14 d the plants resumed growth as demonstrated by an increase in length of the main stem.
125 During the recovery period no NaCl was added to the nutrient solution and seedlings were
126 covered with transparent plastic pots to keep the relative humidity of the air above 90%. After
127 the recovery period salt treatments were started. Plants were randomly allocated into 12
128 treatment groups, consisting of 0, 5, 50, 100, 150, 200, 250, 300, 400 and 500 mM NaCl and two
129 seawater treatments with EC values of 20 and 40 dS/m (seawater was collected at Bergen aan
130 Zee, The Netherlands, March 2011). Extra nutrients were added (KNO_3 85.5 mg L^{-1}, $NH_4H_2PO_4$
131 3.3 mg L^{-1} and Fe-EDDHMA 5 mg L^{-1}) to the two seawater treatments to prevent shortage of
132 essential nutrients. Each treatment group consisted of 9 replicate plants. NaCl treatments were
133 applied by adding NaCl to the nutrient solution in steps of 50 mM per day to prevent an osmotic

shock (Munns 2005), salinity in plants subjected to the seawater treatments was also increased daily with the equivalent (5 dS/m) of 50 mM NaCl. Nutrient solutions were changed weekly. Plants were grown in a randomized design in a naturally lit greenhouse with additional lamps (PAR of 300 µmol m^{-2} s^{-1} at plant level, 14/10 h light/dark) in March-May 2011 in Amsterdam, The Netherlands, maximal outdoor light intensity was PAR of ~1500 µmol m^{-2} s^{-1}. The temperature was 20±2/16±2 ºC day/night, and the relative humidity of the air 70±10/90±2 % day/night, respectively. Pots were randomly re-located every week. The duration of the treatment was 42 d.

Non-destructive growth measurements. During the experiment growth was followed weekly non-destructively as increase of main stem length, number of internodes of the main stem, number of branches and thickness of the 3rd succulent internode from the shoot base measured with a thickness gauge (NO. 2046-08, accuracy 0.01 mm, Mitutuyo, Japan).

Harvest. Plants were harvested after the recovery period before the start of the salt treatment (initial harvest) and after 42 d of the start of the salt treatment. Plants allocated to the 0 and 5 mM NaCl treatments started to flower 14 d after commencement of treatment, and were harvested 21 d after the start of the treatment due to retarded growth, indicated by a light brown yellowish color and wilting. At harvest plants were rinsed in de-mineralized water 3 times for 5 s and carefully blotted dry. Thereafter, the plants were separated into succulent leaf tissue, woody leaf tissue (i.e. not succulent) and roots. Note that because of the reduced growth form of *Salicornia* the words leaf and stem are interchangeable in this paper. Succulent leaf tissue was further divided into expanding succulent leaf tissue, assumed to be the first 1-4 mm from the tips (apexes) of the stems, and expanded succulent leaf tissue, assumed to be from 4 mm of the tips of the stems onward (away from the apexes). Separating expanding and expanded shoot tissue

was done by cutting with a razor blade. Fresh biomass of different shoot tissues and roots were recorded and total leaf area of the green tissues was estimated with a leaf area meter (LI-COR 3100, Li-Cor inc., Lincoln, NE, USA). From a subgroup of plants (3 plants per treatment) diameter and length of all individual branches were recorded and leaf area calculated. Leaf area measurements with the leaf area meter gave a consequent lower value for leaf area than the leaf area calculations on the basis of diameter and length; this is because of the cylindrical growth form of the plants. Therefore a correction for leaf area determined by the diameter-length method was applied on the leaf area values measured with the leaf area meter. The reason to still use the leaf area meter is that the diameter-length measurements are more laborious and time consuming due to the many branches the plant develops. After measuring leaf area, subsamples of shoot and root tissues were shock-frozen in liquid nitrogen and either stored at -20ºC, -80ºC or freeze-dried for 5 d. Other subsamples were oven dried at 70 ºC for 72 h after which dry masses were recorded. Dried plant material was ground into a fine powder using mortar and pestle. Material stored at -20ºC was used to measure osmotic potential. Freeze-dried material was used to analyze glycine betaine. Oven-dried material was used to measure Na^+, Cl^-, K^+ and Mg^{2+} and to calculate the RGR, with the formula (Hunt et al., 2002):

$$RGR = \frac{Ln(w_2) - Ln(w_1)}{t_2 - t_1}$$

Where, W_1 and W_2 is the dry weight or ash-free dry weight of the plants at initial and final harvest, respectively, and t_2-t_1 is the time in days between the two harvests.

Carbon isotope composition. Measurements of carbon isotope composition were made on subsamples of oven-dried expanding and expanded leaf tissue and were determined using an elemental analyzer (NC2500, ThermoQuest Italia, Rodano, Italy) coupled online to a stable isotope ratio mass spectrometer (Delta plus, ThermoQuest Finnigan, Bremen, Germany). To

check for the reproducibility of the method a plant sample (-28.50 ‰; 45.2 % C) was measured in triplicate in each run. Expanding and expanded leaf tissue samples were measured in duplicate. We expressed stable isotope abundance using the δ notation:

$$\delta^{13}C\ (‰) = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000$$

In this formula, $\delta^{13}C$ is the ^{13}C content and R_{sample} and $R_{standard}$ refer to the $^{13}C/^{12}C$ -ratio of the sample and of the standard (Vienna PeeDee Belemnite), respectively.

Analyses of Na^+, Cl^-, K^+, Mg^{2+}. Expanding leaf tissue, expanded leaf tissue, woody tissue and roots were measured for Na^+, Cl^-, K^+ and Mg^{2+} contents. Material was prepared by heating (~100 °C) 50 mg of plant material in 5 ml de-mineralized water for 2 h, hereafter material was filtered with 4-7 µm cellulose filters (597, Whatman GmbH, Dassel, Germany). Na^+, K^+ and Mg^{2+} concentrations were determined in the supernatant on a flame atomic spectrophotometer (Perkin-Elmer 1100B; Perkin Elmer Inc., Waltman, MA, USA). Chloride was measured with a chloride analyzer (Chloride analyzer 926, Sherwood Scientific Ltd, Cambridge, UK).

Glycine betaine. Glycine betaine concentrations were determined in subsamples of shoot and root tissue using high performance liquid chromatography (HPLC) by the method of Carillo and Gibon (2011). Before analyses samples were filtered over 0.22 µm nylon centrifuge filters (Costar Spin-X, Corning Inc., NY, USA) and analyzed by injection of 100 µl of sample into the HPLC (Waters 2690 Alliance system equipped with two Nova-Pak C18 columns (150 mm length * 3.9 mm internal diameter) and a Nova-Pak C18 guard column with a Waters 996 photo diode-array detector; Waters Corporation, Milford, MA, USA). Reliability of the method was

checked by injection of known amounts of glycine betaine (obtained from Sigma-Aldrich, Germany) into tissue samples.

Sap osmotic potential. Plants samples were shock-frozen in liquid nitrogen immediately after harvest and stored at -20 ºC in plastic vials. The material was crushed, left to thaw and spun for 30 s at 13000 rpm (Micro Centaur, Beun De Ronde, Abcoude, The Netherlands) while still in the vial just before measurement. 10 µl of the supernatant was used to measure osmolality using a vapour pressure osmometer (Wescor model 5500, Wescor Inc., Logan, UT, USA). Calibration of the osmometer was checked every 10th sample. Samples were measured in duplicate. The osmotic potential (ψ_s) was determined according to the van't Hoff equation: $\psi_s = -c \cdot R \cdot T$

Where, *c* is the osmolality of the solution (osmol L^{-1}), R is the universal gas constant (kg mol^{-1} K^{-1}) and T is the temperature (°K). Ion activity coefficients of root medium and tissue were assumed to be 0.92 (Robinson and Stokes 1959).

2.3 Experiment 2: Outside growth experiment

Plant cultivation and growth measurements. We used a closed seawater drip irrigation system located at the island of Texel at the NIOZ harbor (53°00'N and 4°47'E) in The Netherlands. In May 2011, seeds of the same population of *S. dolichostachya* as in experiment 1 were sown onto coarse sand in wooden crates of 1 m^2 and 20 cm height. Plants were not watered for two weeks after sowing but received sufficient rain water for germination. Treatment was started 2 weeks after sowing by drip irrigation with dilutions of Waddensea water: 0-10-20-30 and 40 dS/m, with added nutrients (KNO_3 85.5 mg L^{-1}, $NH_4H_2PO_4$ 3.3 mg L^{-1} and Fe-EDDHMA 5 mg L^{-1}). Drip irrigation water was applied for 30 minutes every hour during 15 hours a day. The distance between the holes in the irrigation tubes was 30 cm. Holes in the bottom of the

crates allowed drainage of the irrigation water, which was collected in a second crate and returned to the main reservoir with a volume of 1000 L. Reservoirs were completely changed at least every 2 weeks and more often if the electrical conductivity (EC) of the solutions deviated more than 10% from the intended EC. Precipitation was measured with a rain gauge. There were 8 replicate crates for the 10, 20 and 40 dS/m treatment and 4 replicate crates for the 0 and 30 dS/m treatment. An initial harvest of the plants was done at the start of the treatments and 2nd and 3rd harvests were done after 27 and 67 d after commencement of the treatment, respectively. At harvest, plants were rinsed in de-mineralized water for 3 times 5 s and carefully blotted dry. Fresh masses of leaves and roots were recorded and leaf area was measured as described in experiment 1. After measuring leaf area plants were oven dried at 70 ºC for 72 h after which dry masses were recorded. RGR was calculated over the 40 d period between the second and third harvest with the formula described in experiment 1.

2.4 Statistical analyses

Before analysis normality and homogeneity of the data was checked. One-way ANOVA with Tukey's *post hoc* test was used to assess the effect of salt treatment on the different parameters measured. If the normality or homogeneity assumptions were violated, data were either transformed or a non-parametric test (Kruskal-Wallis) was used. We analyzed the effects of salinity on carbon isotope discrimination, osmotic potential, K^+-selectivity and Na^+:K^+-ratio in different tissue types (expanding and expanded plant tissues) with mixed model regression analysis, with individual measurements nested within measurement series and plant identity. Tissue type and salinity level were treated as fixed factors and measurement series and plant identity as random factors. Data analysis was performed using SPSS 17.0.

247

248 **3. Results**

249 *3.1 Growth*

250 The results of the final harvest of the 13 weeks old plants (0 and 5 mM NaCl treatments)
251 and 16 weeks old plants (50-500 mM NaCl treatments) are shown in figure 1. Growth was
252 measured as differences in fresh mass, dry mass and ash free dry mass between treatments.
253 Plants subjected to the 0 and 5 mM NaCl treatments where harvested 21 d after start of the
254 treatment. Shoot and root fresh mass, dry mass and ash free dry mass at 0 and 5 mM NaCl
255 treatments did not differ significantly after three weeks of growth (data not shown) in these
256 plants. In the 50-500 mM NaCl treatments shoot fresh mass (Fig. 1A) and dry mass (Fig. 1B)
257 increased between 50-300 mM NaCl and declined at 300-500 mM NaCl. Shoot fresh mass and
258 dry mass at 300 mM NaCl, was significantly higher compared to the lowest (50 mM) and highest
259 (500 mM) salt treatments. Shoot dry mass at 50 and 500 mM NaCl was 48 and 50% respectively
260 of the dry mass in the 300 mM treatment. Shoot ash free dry mass (Fig. 1C) showed almost the
261 same pattern as for dry mass, except that 400 mM NaCl in the root medium also resulted in a
262 significant decrease in ash-free dry mass. Root fresh mass showed a significant effect of
263 increased salinity (Fig. 1A), 400 and 500 mM NaCl in the root medium decreased root fresh
264 mass by 45 and 48% respectively, in comparison with the 300 mM NaCl treatment. In contrast to
265 the shoots, the salt treatments had no effect on root dry mass (Fig. 1B) or root ash-free dry mass
266 (Fig. 1C) at 50-500 mM NaCl over the 42 d.

267

268 *Figure 1 near here*

269

The RGR calculated on a total plant dry mass basis and on ash-free dry mass basis (Table 2) showed similar results as the shoot dry mass and shoot ash-free dry mass production with the maximum value at 300 mM. The RGR at 300 mM NaCl in the root medium was significantly higher compared to the rate at 50 and 500 mM NaCl on dry mass basis (respectively 82% and 81%), and also significantly higher compared to the RGR at 50, 400 and 500 mM NaCl on a ash-free dry mass basis. The RGR values from the greenhouse hydroponic culture experiment calculated on a dry mass basis where compared with the RGR values of plants grown in the inland seawater drip-irrigation system at the island of Texel for 40 d during the period July–August 2012. These values ranged between 0.063±0.005 and 0.092±0.004 g g^{-1} d^{-1}, 0 dS/m and 40 dS/m salinity in the root zone respectively, and are in the same range as the RGR values of the greenhouse hydroponics experiment.

Table 2 near here

3.2 Water content, succulence and stem diameter

Water content of succulent leaf tissue was calculated as the difference between fresh and dry mass and is expressed on a dry mass basis (Fig. 2A). The water content remained constant up to 400 mM NaCl in the root medium, there was a trend towards a decrease in water content at 500 mM NaCl in the root zone (not statistically significant, p=0.071). Measurements of succulence and stem diameter (Fig. 2B) showed the highest values at 300 mM NaCl in the root medium. Differences in succulence were statistically significant ($p<0.05$), differences in stem diameter were not statically significant (p=0.074) between the different salinity treatments.

Figure 2 near here

3.3 Carbon isotope discrimination

Values of carbon isotope discrimination were measured in expanding and expanded leaf tissues (Fig. 3). Values of $\delta^{13}C$ significantly increased (e.g. less negative values) in expanding and expanded leaf tissue with increasing NaCl concentrations in the root medium. The interaction between NaCl treatment and carbon isotope discrimination did not differ significantly between the two tissue types ($p=0.066$).

Figure 3 near here

3.4 Leaf sap osmotic potential

Molarity of Na^+, Cl^- and K^+ of expanding and expanded leaf tissues (Table 3) was calculated from tissue ion data and expressed on a water basis as the difference between fresh and dry mass. Contributions of Na^+ and Cl^- to the leaf tissue sap osmotic potential (Table 3) were calculated from the tissue molarity data, assuming that Na^+, Cl^- and K^+ in the plant are osmotically active with an osmotic coefficient of 0.92 along the salinity range 50-500 mM NaCl (Robinson and Stokes 1959). Na^+ and Cl^- were estimated to account for 66-75% of the sap osmotic potential of expanding leaf tissue and for 68-83% of the sap osmotic potential of expanded leaf tissue.

The osmotic potential of expressed leaf sap significantly decreased as the osmotic potential of the solution decreased (Fig. 4). The difference between osmotic potential of sap of

315 expanding and expanded leaf tissue and the osmotic potential of the root medium significantly
316 decreased from 1.574 and 1.521 MPa, respectively, at 50 mM NaCl in the root medium to 0.636
317 and 0.744 MPa, respectively, at 500 mM NaCl in the root medium. The osmotic potential of both
318 tissue types reacted in the same way to the NaCl treatments.
319
320 *Table 3 and figure 4 near here*
321
322 *3.5 Glycine betaine*
323 Concentrations of glycine betaine expressed on dry mass basis ranged between 343 and
324 486 μmol g^{-1} dm for succulent leaf tissue and between 168.5 and 275.9 μmol g^{-1} dm for roots, at
325 150 and 500 mM NaCl in the root medium, respectively (data not shown). Expressed on a water
326 basis (the difference between fresh and dry mass), glycine betaine concentrations were between
327 29.3 and 44.7 mM in the succulent leaf tissue and between 25.4 and 35.1 mM in the roots (Table
328 4) and not changing with increasing salinity, although there was a trend (p=0.054) towards
329 higher concentrations of glycine betaine in succulent leaf tissue at 500 mM NaCl compared to
330 the plants grown at 100-400 mM NaCl in the root medium.
331
332 *Table 4 near here*
333
334 *3.6 Tissue ions*
335 Concentrations of Na^+, Cl^-, K^+, and Mg^{2+} were determined in roots, woody leaf tissue,
336 and expanding and expanded leaf tissue. Na^+, Cl^-, K^+ concentrations expressed on a water basis
337 in expanding and expanded leaf tissue are shown in table 3, concentrations in woody leaf tissue

and roots are not shown. Na^+ and Cl^- significantly increased in all of these tissues when NaCl concentrations in the root medium were increased. In contrast to Na^+ and Cl^-, concentrations of K^+ decreased in all tissues when NaCl concentrations in the root medium were increased from 50 to 150 mM NaCl and remained fairly stable when concentrations were further increased from 150 to 500 mM NaCl in the root medium. Concentrations of Mg^{2+} significantly decreased in succulent leaf tissue and root tissue when NaCl concentrations in the root medium were increased from 50-150 mM NaCl (data not shown). Mg^{2+} concentrations in woody leaf tissue did not change with increasing salinity in the root medium.

The Na^+:K^+-ratio of expanding and expanded leaf tissue significantly increased upon increasing NaCl concentrations in the root medium (Fig. 5A). There was a significant interaction between treatment and tissue type ($p<0.05$). Selectivity for K^+ over Na^+ in the plant was calculated as the ratio of K^+ concentrations in expanding or expanded leaf tissue to that of the root medium, divided by the ratio of Na^+ concentrations in expanding or expanded leaf tissue in the plant to that of the medium (Fig. 5B). K^+-selectivity (S_{K^+,Na^+}) significantly increased in both tissues with increasing NaCl concentrations in the root medium. The interaction effect between the S_{K^+,Na^+} in the two tissue types upon NaCl concentrations in the root medium was significantly different.

Figure 5 near here

4. Discussion

Growth

The present study shows that growth of *Salicornia dolichostachya* increased when salt concentrations in the root medium were increased from 50 to 300 mM NaCl (Fig. 1). Optimal growth occurred at 300 mM NaCl, and growth declined when concentrations were further increased to 500 mM NaCl. The basis upon which growth is expressed in succulent halophytes can be deceptive. If growth is expressed on fresh mass or dry mass basis, a high water content and a large contribution of inorganic ions; Na^+, Cl^-, K^+ (i.e. ash), can obscure an organic growth effect (Yeo and Flowers, 1980). Optimal growth expressed as dry mass at concentrations of 50-250 mM NaCl in the root medium has been observed for a substantial number of dicotyledonous halophytes, to a lesser extent growth stimulation of organic dry mass has been reported (cf. Flowers and Colmer, 2008; Flowers et al., 1986). The growth optimum at 300 mM NaCl in the root medium in this study was the same regardless of growth expression on fresh mass, dry mass or ash-free dry mass basis, indicating that the observed growth stimulation is not merely based on inorganic ion accumulation. This has also been previously reported for *S. bigelovii* (Ayala and Oleary, 1995). Nevertheless, the contribution of ash to shoot dry weight increased gradually with increasing NaCl in the root medium, resulting in lower values of ash-free dry mass at higher (400-500 mM NaCl) salt concentrations in the root medium (Fig. 1C). Regardless of large contributions of inorganic ions to dry mass, most salinity growth experiments performed with *Salicornia* spp. only report growth expressed on a dry mass basis. We summarized the literature data on growth response (expressed on dry mass basis) of *Salicornia* spp. to external salinity (Table 1). Of the 20 papers included in table 1 (for a description of the criteria for literature search see caption of Table 1), 16 papers reported higher growth of plants when the salinity of the external medium was higher than the salinity of the control treatment, the latter being defined as less than 10 mM NaCl in the root medium. In contrast, four papers did not find higher growth

upon salt addition in the root medium at all. The reported growth stimulation by NaCl differed in extent; between 102 and 405% of the control dry weight, and occurred at different external salt concentrations; ranging from 85 to 600 mM NaCl. There were, as well, large differences in absolute values of the recorded dry masses between the papers even referring to the same species within the genus *Salicornia* e.g.; absolute dry mass values ranged from 2.3 mg plant^{-1} for *S. europaea* at 200 mM NaCl in the root medium for 45 d (Aghaleh et al., 2009) to 13.8 g plant^{-1} for *S. europaea* at 300 mM NaCl external salinity grown as well for 45d (Balnokin et al., 2005). Treatments started at germination or 14 d after germination, respectively.

Table 1 near here

The taxonomy of *Salicornia* spp. is described as notoriously difficult (Davy et al., 2001; Kadereit et al., 2007) with large phenotypic differences between the same species and morphological parallelism between different species, which can readily lead to wrong identification of the species. Identification of *S. dolichostachya* in this study was established based on the morphological features described in Huiskes et al. (1985) and confirmed by one of the authors of this article. Also, the collection of seeds from the solitary plants at the lowest locations in the salt marsh gave additional support that the species used in this study is most likely *S. dolichostachya*. As might be expected, environmental factors also influence the shape and height of the growth response curve (Barbour, 1970), such as, nitrogen availability (Rozema 1987), availability of other minerals in the culture medium (e.g., Ca^{2+}, Munns and Tester, 2008) and light intensity (Ushakova et al., 2008). Furthermore, differences in the experimental treatments influence the recorded growth (rate), e.g. the duration of the treatments and age of the

406 plants at the start of the treatment because differences in growth between salinity treatments
407 become more pronounced with increasing time. Another reason for differences between studies
408 may arise from the number and size of the interval in NaCl concentrations in the treatments; a
409 limited number of concentrations and wide intervals allow only a coarse indication of the
410 response curve. Finally, another complicating factor with comparing growth curves of species
411 between different experimental setups lies in the choice of an appropriate control. In this study,
412 the plants at 0 and 5 mM NaCl in the root medium started to flower, ceased to grow and looked
413 unhealthy (indicated by a light brown yellowish color and wilting) 14 d after the start of the salt
414 treatment. The plants received only trace quantities of NaCl present in the potting soil during the
415 first 61 days of the pre-cultivation period, 14 d before start of the salt treatment they were grown
416 on water culture with 0.01 mM Na^+. Poor survival of *S. bigelovii* at low Na^+ concentrations was
417 also found by Webb (1966). In contrast, numerous coastal plants demonstrate optimal growth at
418 0 mM NaCl in hydroponic culture (Rozema and van Diggelen, 1991). In the literature, it has
419 been concluded that there is no obligatory salt requirement for growth of dicotyledonous
420 halophytes (Flowers et al., 1977). Except for, plants with a C_4-photosynthetic pathway that
421 requires small amounts (< 0.1 mM) of Na^+ for regeneration of phosphoenolpyruvate (Brownell
422 and Crosslan, 1972). Although, use of NaCl-free solutions can be regarded as deficient for
423 dicotyledonous halophytes that have optimal growth at NaCl concentrations above 50 mM
424 (Subbarao et al., 2003), as for *S. dolichostachya* in this study. Does impaired growth of
425 *Salicornia* plants in 0-5 mM NaCl then reflect a NaCl requirement of *Salicornia* over a
426 prolonged time period? And if so, which part(s) of the metabolism of *Salicornia* plants require(s)
427 NaCl? To our knowledge this question remains unanswered. NaCl concentrations in the
428 environments where *Salicornia* spp. naturally occur generally range from approximately 250

mM in early spring to as much as 750 mM in autumn (Mahall and Park, 1976), and were found to be seldom lower than 20 mM (Silva et al., 2007). A good control would be to choose a NaCl concentration towards the lower values in this range which is still sufficient for the plants to sustain minimal growth. Overall, comparing growth curves from different experiments requires a standardized way of conducting growth experiments and expressing growth.

It has been suggested that as a consequence of salt tolerance halophytes might be slow growing (Niu et al., 1995). To investigate if a trade–off between salt tolerance and relative growth rate (RGR) exists, we measured the RGR (Table 2) and compare it to RGR values for a variety of plants species in the literature. In this study, the highest RGRs, 0.092 g g^{-1} d^{-1} and 0.080 g g^{-1} d^{-1} expressed on dry mass and ash-free dry mass basis, respectively, coincide with the optimal external salinity (300 mM NaCl) as was found for growth expressed on fresh mass, dry mass and ash-free dry mass basis (Fig. 1). The RGR values of the outside seawater drip-irrigation system were compared to the RGR values found in the hydroponic greenhouse experiment (Table 2). These RGR values were in the same range, which indicates that growth in the greenhouse experiment was not restricted by e.g. light intensity and relative humidity compared to the outside growth experiment. RGR values expressed on ash-free dry mass basis were lower than RGR values expressed on dry mass basis, but showed the same pattern. Only a few of the previous studies conducted with *Salicornia* spp. in hydroponic culture reported RGR values. The highest RGR values reported (expressed on an ash-free dry mass basis) were for *S. bigelovii*; 0.160 g g^{-1} d^{-1} at 200 mM NaCl in the root medium (Ayala and Oleary, 1995) and for *S. europaea*; ~0.3 g g^{-1} d^{-1} during 7 d at 0 mM NaCl after 21 d of growth at 540 mM NaCl (Glenn and Oleary, 1984). If we compare these RGRs and the RGR of *S. dolichostachya* in this study to the potential RGRs found in a comparison of over 100 wild plant species (though not in

452 relation the salinity (Grime and Hunt, 1975)), the RGRs of *Salicornia* spp. lie towards the middle
453 (*S. dolichostachya*, this experiment) or higher range (*S. bigelovii*, Ayala and Oleary, 1995; *S.*
454 *europaea*, Glenn and Oleary, 1984) of the RGR values in the comparison of Grime and Hunt
455 (1975). Grime and Hunt (1975) reported RGRs between 0.05 g g^{-1} d^{-1} and 0.3 g g^{-1} d^{-1}. Reports
456 on the yield of halophytes (a.o. *S. bigelovii*) under saline field conditions listed yields that were
457 comparable with conventional crops (cf. Glenn et al., 1999), which shows the potential use of
458 *Salicornia* in saline agriculture.
459
460 *Morphological and physiological aspects of salt tolerance in S. dolichostachya*
461 Leaf succulence (water content per unit leaf area) and leaf diameter in *S. dolichostachya*
462 both showed the highest values at 300 mM NaCl in the root medium (Fig. 2), this coincides with
463 the NaCl concentration at which optimal growth is observed in this experiment. Increase in leaf
464 succulence and leaf stem diameter may be achieved by an increase in size of the cells and the
465 relative size of their vacuoles or an increase in the number of cell layers (Flowers et al., 1986;
466 Shabala and Mackay, 2011). It has been suggested that an increase in thickening of the leaf
467 tissues in response to salinity is a direct response to have increased storage area for ions (i.e. Na^+
468 and Cl^-) (Jennings, 1968) and to reduce the surface area which leads to increased water use
469 efficiency.
470 Carbon isotope discrimination is often related to stomatal conductance; more carbon
471 isotope discrimination occurs when stomata are open. In this study, carbon isotope
472 discrimination decreased (i.e. $\delta^{13}C$ values increased) linearly with increased salinity in the root
473 medium (Fig. 3), indicating stomatal closure at higher salinities. Open stomata facilitate water
474 loss and at the same time discrimination against $^{13}CO_2$ is high. Lower discrimination against ^{13}C

475 is correlated with closure of stomata (Farquhar et al., 1989) and when this leads to reduced
476 transpiration also with increased long term water use efficiency. Decreased carbon isotope
477 discrimination with increasing salinity has previously been reported for *S. bigelovii*, (Ayala and
478 Oleary, 1995) and for *S. rubra* (Khan et al., 2001). Note that the lowest $\delta^{13}C$ values in Fig. 4 (at
479 500 mM NaCl in the root medium) do not coincide with the highest values for succulence and
480 stem diameter (at 300 mM NaCl), although these three factors are all thought to be correlated
481 with water use efficiency. As we did not measure plant transpiration we are unable to calculate
482 water use efficiency as the ratio of biomass increase per unit water, therefore we do not know
483 how water use efficiency changes with increasing salinity.

484 One of the biggest challenges of growth in a saline environment is to assure water
485 absorption. This means that plants have to adjust their tissue water potential to a level that is
486 lower (e.g. more negative) than that of the soil water. The total water potential of the plant is
487 described by the formula: $\psi_w=\psi_s+\psi_p$, where; ψ_w is the total water potential, ψ_s is the osmotic
488 potential and ψ_p the turgor pressure. Adjustment of the water potential to a lower (more negative)
489 level can be achieved by osmotic adjustment (a.o. ion uptake), decreased turgor or decreased cell
490 volume. It is generally assumed that in dicotyledonous halophytes osmotic adjustment is the
491 main factor in lowering the water potential, and that this is largely achieved by accumulation of
492 Na^+ and Cl^- in the vacuole (Gorham et al., 1980, Flowers et al., 1977; Greenway and Osmond,
493 1972). In this study, *S. dolichostachya* was able to keep the difference between the osmotic
494 potential of the leaves more negative than the water potential of the external solution (Fig.3). The
495 contributions of the major ions Na^+ and Cl^- to the osmotic potential increased significantly, with
496 increasing external salt concentrations (table 2). Concentrations of Na^+ and Cl^- were between
497 321-574 mM and 266-631 mM, respectively, in expanding leaf tissue at 50-500 mM NaCl in the

498 root medium (Table 3). In expanded leaf tissue values Na^+ and Cl^- were slightly higher at 500
499 mM NaCl in the root medium. Molality of the cell sap was calculated using the water content
500 and the tissue ion data, assuming that Na^+ and Cl^- are predominantly present in the vacuole
501 (Flowers et al., 1986) and these ions are evenly distributed in cells of different tissues (the latter
502 can be argued; Conn and Gilliham, 2010; Fricke et al., 1996; Karley et al., 2000). Taken together
503 Na^+ and Cl^- contributed between 66-75 % and 68-83% to the measured osmotic potential, in
504 expanding and expanded leaf tissue, respectively. Note that in this study we did not measure
505 water potential, but Ayala and Oleary (1995) showed in a study with *S. europaea* a strong linear
506 relationship between water potential and osmotic potential and no significant effect of turgor
507 pressure over an external salinity range of 5-500 mM NaCl. Supplementary to increased
508 accumulation of Na^+ and Cl^-, concentrating ions in the cell sap due to lower water content, could
509 be an additional mechanism in lowering the water potential at above optimal salinity, as found
510 for *S. europaea* (Glenn and Oleary, 1984).

511 It is generally assumed that storage of Na^+ and Cl^- is restricted to the vacuoles to avoid
512 critical salt concentrations in the cytoplasm (Flowers et al., 1977; Greenway and Osmond, 1972).
513 Other organic solutes, like glycine betaine, are thought to accumulate in the cytoplasm to
514 maintain water potential equilibrium within the cell (Storey and Wyn Jones, 1979), where they
515 might also serve a function in stabilizing enzymes in the presence of salts (Jolivet et al., 1982;
516 Pollard and Wyn Jones, 1979), as ROS scavenger (cf. Bohnert 1995) or as ion channel regulator
517 (Cuin and Shabala, 2007). Glycine betaine is thought to be the main organic osmolyte in
518 *Salicornia* spp. (Gorham et al., 1980) and other species of the family of the Amaranthaceae
519 (Weretilnyk et al., 1989). In this experiment, concentrations of glycine betaine in succulent leaf
520 tissue and roots expressed on dry mass basis are consistent with concentrations previously

reported in the literature (*S. europaea*, Gorham et al., 1980; *S. europaea*, Moghaieb et al., 2004). Glycine betaine concentrations expressed on a dry mass basis differed significantly between treatments in both shoot and root tissue. However, assuming that glycine betaine is dissolved in water in the cell, expressing concentrations on a water basis makes more sense. When expressed on a water basis glycine betaine concentrations in succulent leaf tissue are between 29.3 and 44.7 mM and in roots between 25.4 and 35.1 mM (Table 4). Glycine betaine concentrations did not differ significantly between the treatments, although there was a strong trend towards higher glycine betaine concentrations in leaf tissue at 500 mM NaCl in the root medium ($p=0.054$). Previous studies with dicotyledonous halophytes reported either increased accumulation of glycine betaine with increasing salinity (*S. europaea*, Moghaieb et al., 2004; *A. spongiosa*, Storey and Wyn Jones, 1975) or, as in this study, no correlation between glycine betaine accumulation and increased salinity (*S. monoica*, Storey and Jones, 1975). Supposing that glycine betaine is solely present in the cytoplasm (Hall et al., 1978) and that the volume of the cytoplasm is approximately 10% of the cell volume (Hajibagheri et al., 1984), then glycine betaine concentrations would be 10 fold higher, between 293 and 447 mM and thus osmotically relevant. These glycine betaine concentrations in the cytoplasm would be in a range capable of balancing a substantial part, ~50 % in this study at an external salinity between 50 and 400 mM, of the osmotic potential generated in the vacuole by Na^+ and Cl^-. It is likely that glycine betaine together with other compatible solutes (Gorham et al., 1980; Hasegawa et al., 2000), can generate a sufficient osmotic potential to maintain water potential equilibrium within the cell. Interestingly, is that the glycine betaine concentrations did not change between the different salinity treatments. Therefore, one could argue a primary role for glycine betaine in osmotic adjustment.

High external Na^+ concentrations can lead to K^+ deficiencies in plants (Flowers et al., 1977), because of the great similarity of these monovalent cations in physical and chemical properties. K^+ is regarded as an essential ion for enzyme functioning, whereas only plants with a C_4-photosynthetic pathway require Na^+ as a micronutrient (Brownell and Crosslan, 1972). However, Na^+ can be regarded an essential ion in halophytes like *Salicornia* (discussed above). In this study, the Na^+:K^+-ratio in expanding and expanded leaf/stem cells increased significantly with increasing NaCl concentrations in the root medium over the range of 50-500 mM NaCl (Fig. 5A). The leaf Na^+:K^+-ratios of *S. dolichostachya* are much higher compared to Na^+:K^+-ratios of most species (Flowers et al., 1986). The halophytic species of the Amaranthaceae family together with the members of the Azoceae have generally the highest Na^+:K^+-ratios, which suggest that there must be a specific trait present in these families to be able to sustain such high ratios which are detrimental to most other species. Although absolute values of Na^+ increased and K^+ decreased in *S. dolichostachya*, K^+-selectively (S_{K^+,Na^+}) significantly increased upon increasing NaCl concentrations in the root medium in both expanding and expanded leaf tissue (Fig. 5B). The increase in S_{K^+,Na^+} in expanding leaf tissue was gradual upon increasing salinity, whereas the S_{K^+,Na^+} in expanded tissue increased in general over the salinity range of 50-500 mM NaCl, but reached its lowest values at 250 mM NaCl in the root medium. High K^+ affinity has been reported before in a dicotyledonous halophyte (*Suaeda monoica*, Storey and Wyn Jones, 1979). This increased S_{K^+,Na^+} indicates that *S. dolichostachya* possesses a mechanism to specifically absorb K^+ in the presence of high external Na^+ levels, and that the partitioning of Na^+ and K^+ might vary between different types of leaf tissues.

Conclusions

In this study, *Salicornia dolichostachya* showed growth stimulation at 300 mM NaCl in the root medium. At 50 and 500 mM NaCl in the root medium dry mass was reduced by 48 and 50% respectively in comparison with the dry mass at 300 mM root zone salinity. The growth response curve upon salinity was the same whether expressed on fresh mass, dry mass or ash-free dry mass, although the contribution of ions to the dry mass increased with increasing external salinity. 0 and 5 mM NaCl in the root medium were not sufficient for the plants to maintain growth, suggesting a salt requirement for *S. dolichostachya* for prolonged time periods of growth. No general conclusion based on a comparison between growth response curves of *Salicornia* spp. from the literature could be drawn, due to differences in cultivation and experimental conditions. The highest succulence and thickest stem diameter coincided with the growth optimum at 300 mM NaCl, indicating a correlation between these parameters and salt stimulated growth of *S. dolichostachya*. Stomatal closure (measured as $\delta^{13}C$) and expressed leaf sap osmotic potential gradually increased and decreased, respectively, over the salinity range of 0-500 mM NaCl. Leaf sap osmotic potential was chiefly lowered by accumulation of Na^+ and Cl^-. Glycine betaine concentrations did not change significantly upon salinity. Na^+:K^+-ratio and K^+-selectivity increased over the salinity range and differed between expanding and expanded leaf tissue, suggesting partitioning of Na^+ and K^+ between different tissue types. Future growth evaluations should be done under standardized conditions and need to be conducted at the tissue or cellular level to be able to pinpoint the processes underlying the salt requirement, salt stimulation and salt tolerance of *Salicornia* spp.

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Table legends

Table 1. Effect of salinity on growth of *Salicornia* spp. under a variety of conditions in salt dose response experiments performed up until 1th of August 2011. Literature was searched with the database Web of Science using the search terms: *Salicornia* and salt (434 hits) or *Salicornia* and salinity (196 hits). Studies were only included when they were conducted in either greenhouses or controlled environment rooms, reported dry mass for at least 2 different salt treatment levels of which one control treatment (< 10 mM NaCl) and were written in English. Dry mass data given in graphs were made numeric with DataThief III (Shareware; version 1.6). All dry weight data were systematically expressed in the same unit preferably as plant dry mass, in two studies only shoot dry mass was given. Dry mass at the recorded optimal salinity per study was expressed as percentage of the dry mass at the control in the same study. If plants were grown under a number of different conditions, the conditions that gave the highest dry mass values were chosen.

Table 2. Relative growth rates (RGR) of *Salicornia dolichostachya* grown at varying salinities in hydroponic culture (with Hoagland plus NaCl or diluted seawater) in a greenhouse or in an inland seawater drip-irrigation system on the island of Texel. Growth was measured by increase in dry mass or ash-free dry mass. Plants were grown for 42 d for the hydroponic culture experiment and for 40 d for the drip-irrigation experiment. Values are means ± S.E. of 4-9 replicates. Mean values with different letters indicate significant differences within the same column (Tukey's test, $p<0.05$).

Table 3. Molarity of Na^+, Cl^- and K^+ in expanding and expanded leaf tissue and the contribution of Na^+, Cl^- and K^+ to the osmotic potential of the leaf tissue of *Salicornia dolichostachya*

776 hydroponically grown at 50-500 mM NaCl in the root medium for 42 d. The molarities of Na^+,
777 Cl^- and K^+ were calculated from tissue ion data. Values are means ± S.E. of 5-9 replicates.
778 Kruskal-Wallis p-values were: $p<0.05$ for the ion molarities.

779 **Table 4.** Glycine betaine concentrations expressed on a water basis (mM) in shoot and root
780 tissue of *Salicornia dolichostachya* hydroponically grown with 50-500 mM NaCl in the root
781 medium for 42 d. Concentrations of glycine betaine at commencement of the treatments were
782 14.9 ±1.8 and 15.5 ± 1.9 mM in shoots and roots respectively. Values are means ± S.E. of 3
783 replicates. ANOVA p-values were for: shoots $p=0.054$ and for roots $p>0.05$.

784

785 **Figure legends**

786 **Figure 1.** Effect of NaCl treatments on (A) fresh mass (B) dry mass and (C) ash-free dry mass of
787 shoots and roots of *Salicornia dolichostachya* hydroponically grown at 50-500 mM NaCl in the
788 root medium for 42 d. Values are means ± S.E. of 7-9 replicates. Mean values with different
789 letters indicate a significant difference in the same tissue between treatments (Tukey's test,
790 $p<0.05$). No significant differences occurred between treatments in (B) root dry mass and (C)
791 root ash-free dry mass.

792 **Figure 2.** Effect of NaCl treatments on (A) leaf water content (B) leaf succulence and stem
793 diameter (3^{nd} succulent internode from the shoot base) of *Salicornia dolichostachya*
794 hydroponically grown at 50-500 mM NaCl in the root medium for 42 d. Values are means ± S.E.
795 of 7-9 replicates. ANOVA p-values were for: (A) leaf water content $p=0.071$, (B) succulence
796 $p<0.05$, and stem diameter $p=0.074$. Note that the scales on the y-axis are different.

797 **Figure 3.** Effect of NaCl treatments on carbon isotope discrimination in expanding and expanded
798 leaf tissue of *Salicornia dolichostachya* hydroponically grown at 50-500 mM NaCl in the root
799 medium for 42 d. Values are means ± S.E. of 7-9 replicates. Mixed model regression analysis
800 showed: treatment $p<0.05$, and tissue type • treatment $P=0.066$.

801 **Figure 4.** Effect of NaCl treatments on osmotic potentials of expanding and expanded leaf tissue
802 of *Salicornia dolichostachya* hydroponically grown at 50-500 mM NaCl in the root medium for
803 42 d. Values are means ± S.E. of 7-9 replicates. Mixed model regression analysis showed:
804 treatment $p<0.05$, and tissue type • treatment $P>0.05$.

805 **Figure 5.** Effect of NaCl treatments on (A) Na^+:K^+-ratio and (B) K^+-selectivity of expanding and
806 expanded leaf tissue of *Salicornia dolichostachya* hydroponically grown at 50-500 mM NaCl in

807 the root medium for 42 d. Values are means ± S.E. of 7-9 replicates. Mixed model regression
808 analysis showed in (A) and (B): treatment $p<0.05$, and tissue type • treatment $P<0.05$.
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		Salt treatments		Dry mass at recorded optimal salinity as % of control	Treatment specifications		
Salicornia spp.	Origin plants	Control (mM)	Recorded optimal salinity (mM)		Growth media	Pre-cultivation method (d)	d
S. europaea	Inland salt marsh	< 1	170.0	109	Hydroponic culture in perlite	61	
S. europaea	Inland salt marsh	< 1	200.0	102	Hydroponics	Germination	
S. europaea	Inland salt marsh	< 1	300.0	231	Soil	15	
S. europaea	Inland salt marsh	< 1	170	273	Hydroponic culture in vermiculite	Seedlings	
S. europaea	Coastal salt marsh	< 1	< 1	100[a]	Hydroponics at 50% relative humidity	20	
S. europaea	Coastal salt marsh	< 1	300.0	252	Hydroponics with agar	Plants from the field	
S. europaea	na	< 1	171.0	405	Hydroponics	14	
S. europaea	na	< 1	85	143	Hydroponic culture in perlite	Plants from the field	
S. brachiata	Coastal salt marsh	< 1	200.0	249	Hydroponics at 60% relative humidity	61	
S. ramossissima verdemilho	Coastal salt marsh	< 1	< 1	100	Hydroponic sand culture	Seedlings	
S. ramossissima varela	Coastal salt marsh	< 1	< 1	100	Hydroponic sand culture	Seedlings	
S. rubra	Inland salt marsh	< 1	< 1	100	Hydroponics	1 cm tall plants	
S. rubra	Inland salt marsh	< 1	327	195	Hydroponics	2-3 cm tall plants	
S. bigelovii	Coastal salt marsh	0.5 ppt (equivalent to ~9 mM)	10 ppt (equivalent to ~180 mM)	118[a]	Seawater (aquaculture effluent) in sand	Germination	
S. bigelovii	Coastal salt marsh	5	200	301	Hydroponics at 25 - 50% realtive humidity	48-55	
S. persica	Inland salt marsh	< 1	170.0	109	Hydroponic culture in perlite	61	
S. persica	Inland salt marsh	< 1	100.0	136	Hydroponics	Germination	
S. utahensis	Inland salt marsh	< 1	600.0	146	Hydroponic sand culture	Young seedlings	

S. subterminalis	Sweetwater marsh	< 1	394	151	Seawater in sand, silt and clay mixture	30
S. fruticosa	Coastal salt marsh	< 1	342	262	Hydroponics	Plants from the field

na, not available

[a] percentage calculated from shoot dry mass only

Hydroponics			Drip-irrigation	
	RGR (mg g^{-1} d^{-1})			RGR (mg g^{-1} d^{-1})
NaCl (mM)	Expressed on dry mass basis	Expressed on ash-free dry mass basis	Seawater (dS/m)	Expressed on dry mass basis
50	75 ± 4[a]	68 ± 3[a]	0	63 ± 5[a]
100	82 ± 4[ab]	71 ± 5[ab]	10	90 ± 3[b]
150	84 ± 2[ab]	75 ± 2[ab]		
200	82 ± 3[ab]	73 ± 3[ab]		
20 dS/m (220 mM NaCl)	83 ± 5[ab]	78 ± 3[ab]	20	83 ± 2[b]
250	87 ± 3[ab]	76 ± 3[ab]		
300	92 ± 1[b]	80 ± 2[b]	30	81 ± 3[b]
400	81 ± 2[ab]	69 ± 2[a]		
40 dS/m (440 mM NaCl)	82 ± 4[ab]	76 ± 4[ab]	40	92 ± 4[b]
500	75 ± 3[a]	62 ± 6[a]		

Treatment salinity NaCl (mM)	Expanding shoot tissue				Expanded shoot tissue			
	Molarity (mM)			Na^+, Cl^- and K^+ contribution to Ψs (%)	Molarity (mM)			Na^+, Cl^- and K^+ contribution to Ψs (%)
	Na^+	Cl^-	K^+		Na^+	Cl^-	K^+	
50	321	266	73	74	273	353	132	82

100	319	296	50	67	257	313	78	75
150	298	298	43	56	337	376	51	73
200	345	313	35	62	372	403	61	78
250	356	315	30	64	457	400	33	78
300	386	330	26	64	438	402	33	76
400	504	457	35	78	534	472	38	78
500	574	631	32	77	662	613	43	86

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Treatment salinity NaCl (mM)	Tissue glycine betaine concentration (mM)	
	Shoot	Root
50	38.5 ± 6.1	28.3 ± 4.7
100	30.2 ± 2.9	25.9 ± 7.2
150	29.3 ± 0.9	25.4 ± 3.5
200	32.0 ± 2.6	28.8 ± 3.5
250	35.4 ± 2.7	28.4 ± 1.3
300	32.2 ± 0.5	29.3 ± 8.3
400	33.8 ± 2.9	33.0 ± 2.6
500	44.7 ± 3.1	35.1 ± 2.0

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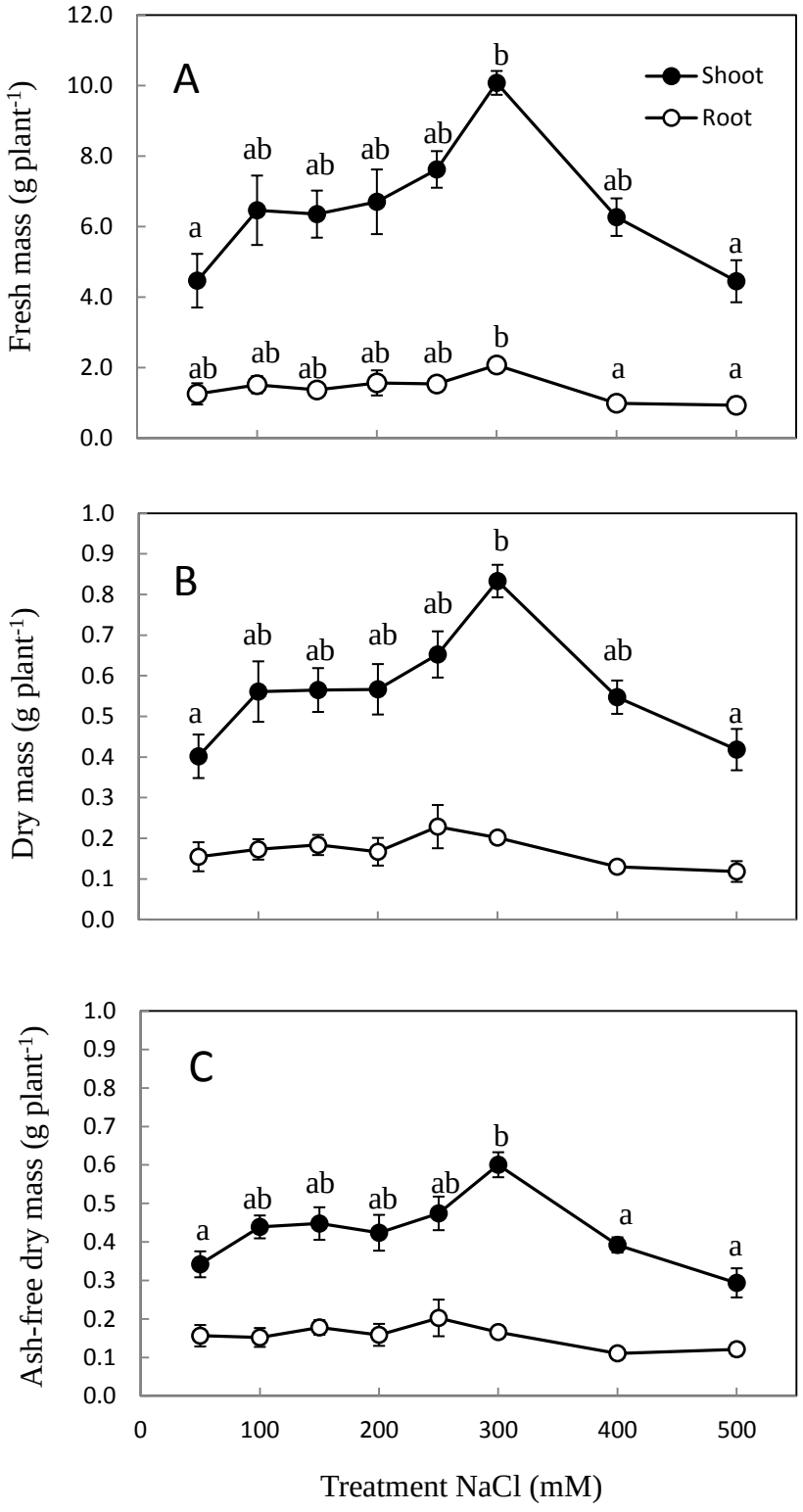
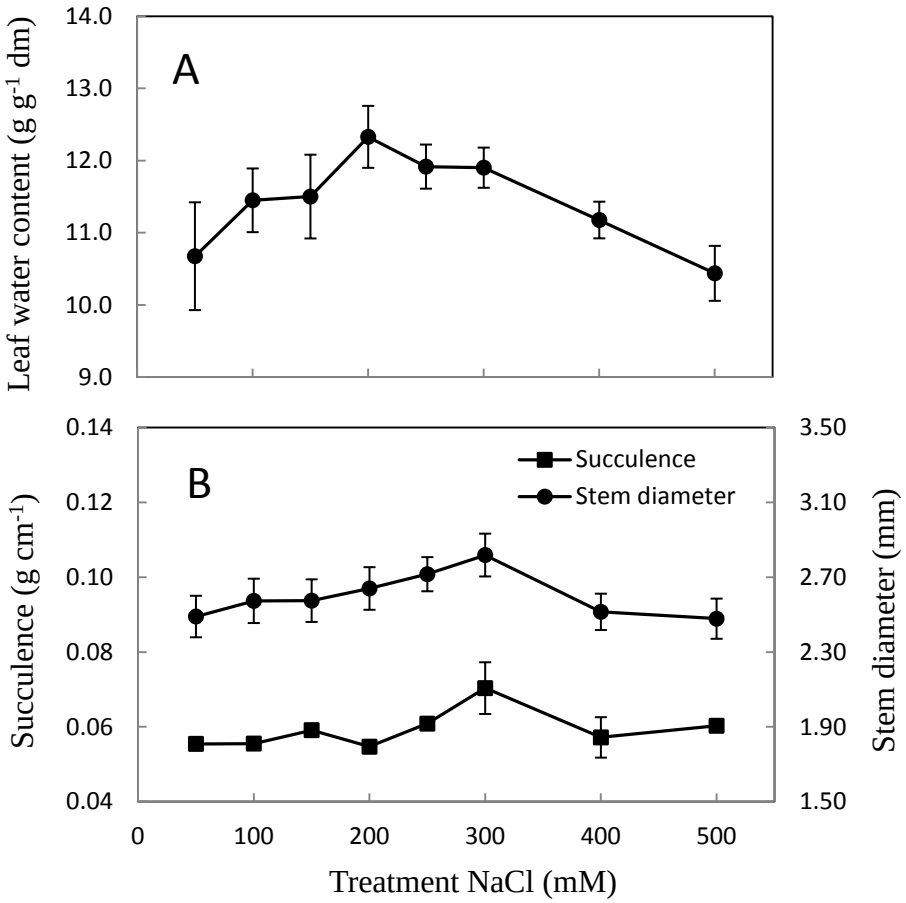
Salicornia dolichostachya Moss in the UNESCO World heritage site 'The Wadden Sea', Wilhelmshaven, Germany.

July, 2011 (Photograph: B. Bruning).

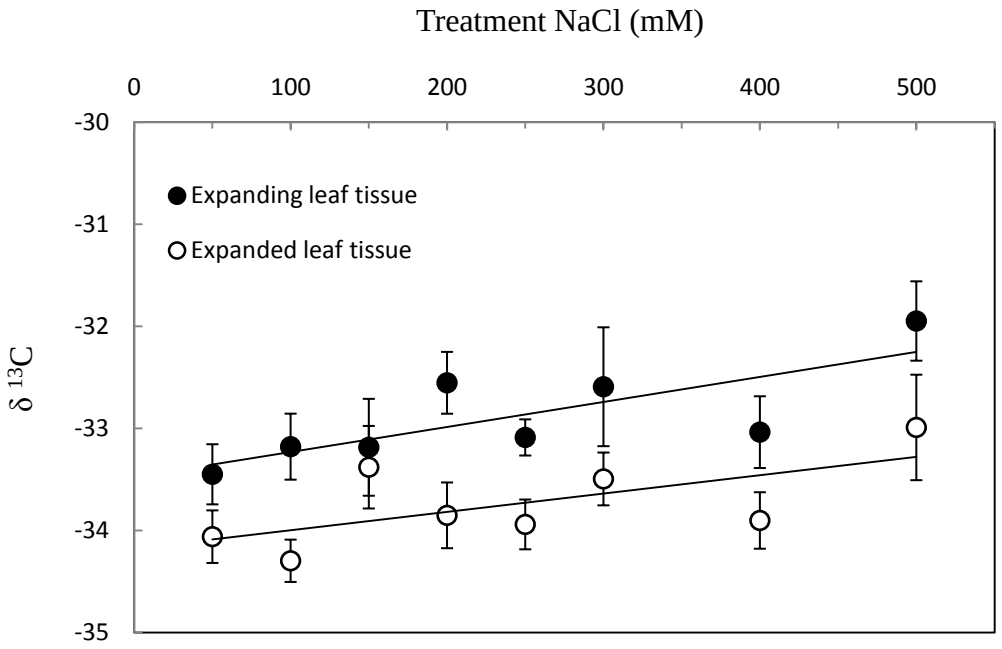
Figure(s)


A
B
C
Shoot
Root
Fresh mass (g plant-1)
Dry mass (g plant-1)
Ash-free dry mass (g plant-1)
Treatment NaCl (mM)
0
100
200
300
400
500
12.0
10.0
8.0
6.0
4.0
2.0
0.0
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
a
ab
b

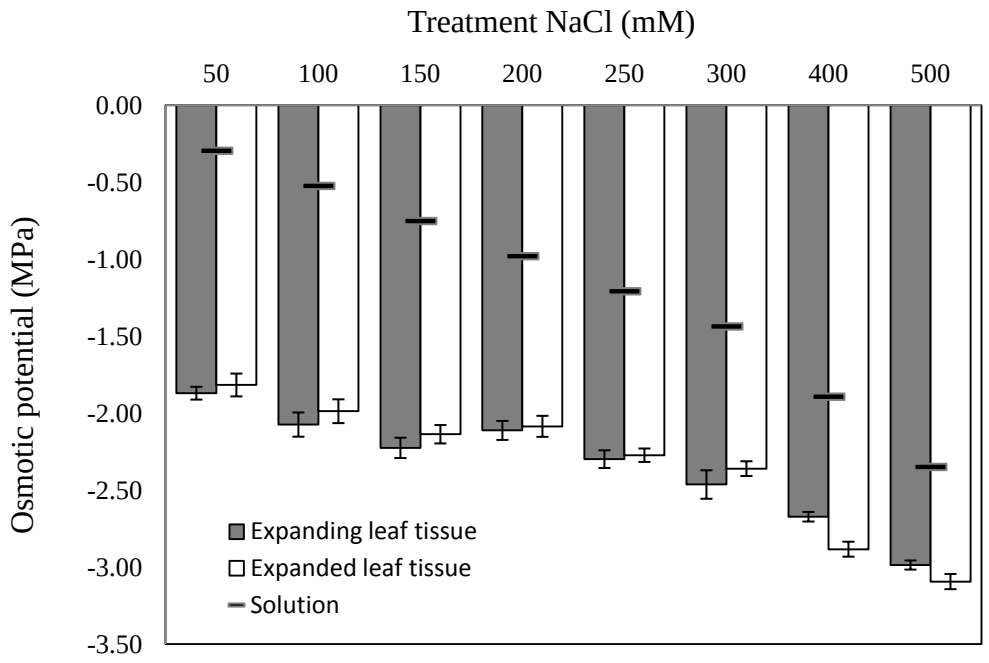
Figure(s)


A
B
Leaf water content (g g-1 dm)
Succulence (g cm-1)
Stem diameter (mm)
Succulence
Stem diameter
Treatment NaCl (mM)
14.0
13.0
12.0
11.0
10.0
9.0
0.14
0.12
0.10
0.08
0.06
0.04
3.50
3.10
2.70
2.30
1.90
1.50
0
100
200
300
400
500

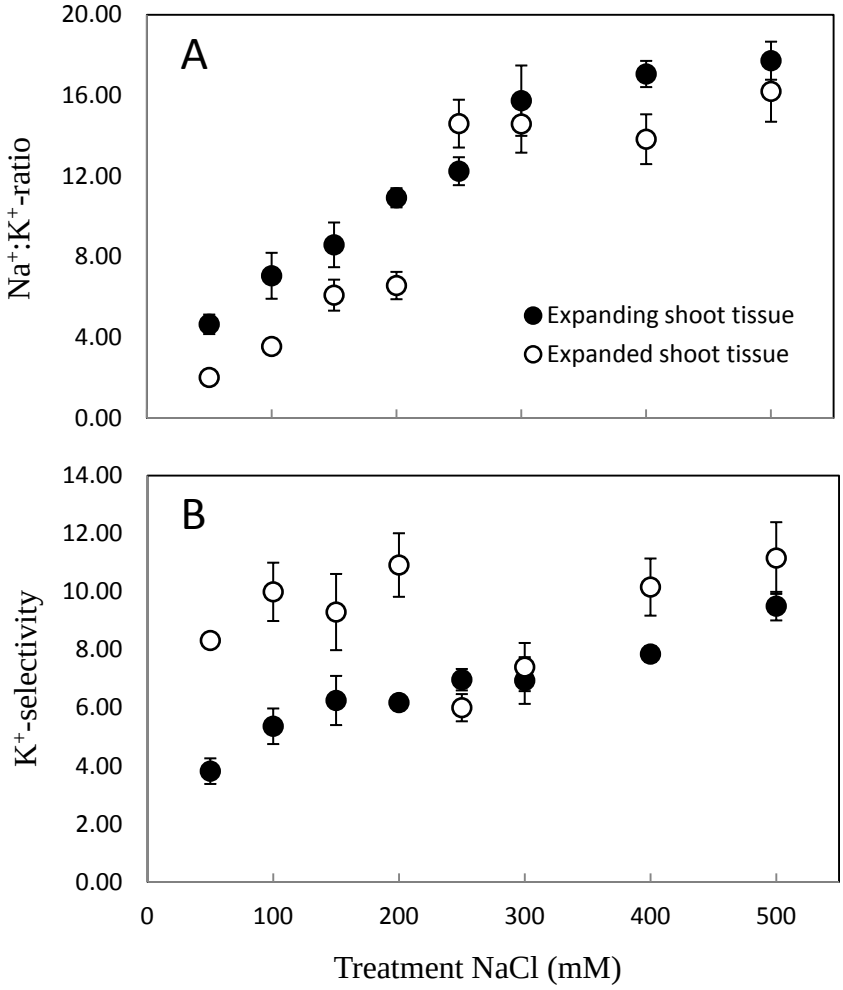
Figure(s)


Treatment NaCl (mM)
0
100
200
300
400
500
-30
-31
-32
-33
-34
-35
δ 13C
Expanding leaf tissue
Expanded leaf tissue

Figure(s)


Treatment NaCl (mM)
50
100
150
200
250
300
400
500
Osmotic potential (MPa)
0.00
-0.50
-1.00
-1.50
-2.00
-2.50
-3.00
-3.50
Expanding leaf tissue
Expanded leaf tissue
Solution

Figure(s)


A
B
20.00
16.00
12.00
8.00
4.00
0.00
14.00
10.00
6.00
2.00
0
100
200
300
400
500
Na+:K+-ratio
K+-selectivity
Expanding shoot tissue
Expanded shoot tissue
Treatment NaCl (mM)

Research highlights

- *Salicornia dolichostachya* had its growth optimum at 300 mM NaCl in the root medium.
- The highest values of succulence and stem diameter coincide with the growth optimum.
- Plants maintained a lower leaf osmotic potential than that of the medium.
- Carbon isotope discrimination decreased linear with increasing external salinity.
- Leaf and root glycine betaine concentrations did not change with external salinity.