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Effect of Nitrate on Root Development and Nitrogen Uptake of *Suaeda physophora* Under NaCl Salinity*[1]

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ABSTRACT

The effects of NaCl salinity and NO_3^- on growth, root morphology, and nitrogen uptake of a halophyte *Suaeda physophora* were evaluated in a factorial experiment with four concentrations of NaCl (1, 150, 300, and 450 mmol L^{-1}) and three NO_3^- levels (0.05, 5, and 10 mmol L^{-1}) in solution culture for 30 d. Addition of NO_3^- at 10 mmol L^{-1} significantly improved the shoot ($P < 0.001$) and root ($P < 0.001$) growth and the promotive effect of NO_3^- was more pronounced on root dry weight despite the high NaCl concentration in the culture solution, leading to a significant increase in the root:shoot ratio ($P < 0.01$). Lateral root length, but not primary root length, considerably increased with increasing NaCl salinity and NO_3^- levels ($P < 0.001$), implying that Na^+ and NO_3^- in the culture solution simultaneously stimulated lateral root growth. Concentrations of Na^+ in plant tissues were also significantly increased by higher NaCl treatments ($P < 0.001$). At 10 mmol L^{-1} NO_3^-, the concentrations of NO_3^- and total nitrogen and nitrate reductase activities in the roots were remarkably reduced by increasing salinity ($P < 0.001$), but were unaffected in the shoots. The results indicated that the fine lateral root development and effective nitrogen uptake of the shoots might contribute to high salt tolerance of *S. physophora* under adequate NO_3^- supply.

Key Words: halophyte, lateral roots, nitrate reductase activity, root morphology

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It is estimated that 1.5 billion ha of land are salt-affected, and soil salinization is a worldwide problem for agricultural and ecosystem conservation (Aiazzi *et al.*, 2002). Saline soils contain extreme ratios of Na^+:Ca^{2+}, Na^+:K^+, and Cl^-:NO_3^-, which causes reduced plant growth due to specific ion toxicities and ionic imbalance acting on biophysical or metabolic components of plant growth (Gratten and Grieve, 1999). The topic of salinity-mineral nutrition relations in halophytes has received less attention than that of glycophytes. Nevertheless, some halophytes, despite their remarkable ability to absorb nutrients selectively from solutions dominated by Na^+ and Cl^-, may also exhibit symptoms of mineral imbalance and disorders (Gratten and Grieve, 1999). Waisel (1985) proposed that the effect of salinity on the growth of halophytes depends on the general water relations of the plants and their nitrogen nutrition. Several studies have demonstrated that application of nitrogen fertilizers stimulated growth of halophytes (Naidoo, 1987; Song *et al.*, 2006). However, the mechanism by which this effect is brought about is not clear (Naidoo, 1987).

For glycophytes, Lacan and Durand (1995) suggested that the primary processes of plant salt tolerance may reside in the roots. There is strong theoretical evidence that roots, in conjunction with the environmental parameters of the shoots, control the salt load to the shoots and consequently are

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directly involved in the whole-plant responses to saline environments (Maggio *et al.*, 2001). Root growth is usually less sensitive to salt stress than shoot growth; therefore, an increased root:shoot ratio is often observed when plants are subjected to saline conditions (Cheeseman, 1988). Salinity-induced changes in the morphology of the root system may have consequences for nutrient uptake (Bernstein and Kafkafi, 2002). However, root growth of halophytes may be affected differently to that of glycophytes.

Past research has focused mainly on processes in shoots, such as compatible osmolyte accumulation (Colmer *et al.*, 1996; Naidoo and Naidoo, 2001), activity of antioxidant enzymes (Misra and Gupta, 2006), and photosynthesis (Kao *et al.*, 2001) under different salinity and nitrogen treatments. Nitrate is not only a nutrient, but can also be a signaling molecule for plant growth (Crawford, 1995; Zhang *et al.*, 1999). Our previous research has confirmed that adequate nitrate supply significantly improves growth of *Suaeda physophora.* This halophyte is widely grown by farmers in Xinjiang, China, because it is a host to parasitic plants, *Cistanche* spp., which is a valuable plant in traditional Chinese medicine (Song *et al.*, 2006). Changes in root system and nitrogen nutrition partitioning between aerial and below-ground parts of halophytes in response to nitrogen fertilization under high salinity have been much less extensively studied (Naidoo, 1987; Liu *et al.*, 2004) than other aspects of halophyte physiology (Flowers and Colmer, 2008). This study aimed to evaluate the interactive effects of nitrate and salinity on root growth and nitrogen uptake of *S. physophora*.

MATERIALS AND METHODS

Seeds of *Suaeda physophora* Pall. were collected from the natural saline habitats of Xinjiang, China, in October 2006. They were germinated on vermiculite in a controlled environment chamber (Conviron-PGR15, Controlled Environment Ltd.,Winnipeg, Canada) with a 12/12 h light/dark cycle at a light intensity (photosynthetically active radiation, PAR) of 480 μmol m^{-2} s^{-1}. The temperature was 30 °C during the day and 20 °C at night and relative humidity was 60%. After 7 d, 12 uniform seedlings were transferred to each 2-L plastic pots filled with a 1 mmol L^{-1} NO_3^- nutrient solution. The NO_3^- nutrient solution contained 0.5 mmol L^{-1} $Ca(NO_3)_2$, 2.5 mmol L^{-1} $CaCl_2$, 1 mmol L^{-1} K_2SO_4, 2 mmol L^{-1} $MgSO_4$, 1 mmol L^{-1} KH_2PO_4, 90 μmol L^{-1} Fe-ethylene diamine tetraacetic acid (EDTA), 46 μmol L^{-1} H_3BO_3, 9.1 μmol L^{-1} $MnCl_2$, 0.32 μmol L^{-1} $CuSO_4$, 0.76 μmol L^{-1} $ZnSO_4$, 0.56 μmol L^{-1} Na_2MoO_4, and 1 mmol L^{-1} NaCl. The pH of the nutrient solution was adjusted to 6.5 ± 0.1 with KOH and H_2SO_4. The nutrient solution was continuously aerated and replaced every two days during pre-culture for 30 d before treatment.

The pre-cultured plants were subjected to 1, 150, 300, and 450 mmol L^{-1} NaCl. At each salinity level, plants were supplied with 0.05, 5, and 10 mmol L^{-1} NO_3^-. The 0.05 mmol L^{-1} NO_3^- nutrient solution contained 0.025 mmol L^{-1} $Ca(NO_3)_2$, 2.975 mmol L^{-1} $CaCl_2$, 2 mmol L^{-1} K_2SO_4, 2 mmol L^{-1} $MgSO_4$, and 1 mmol L^{-1} KH_2PO_4; the 5 mmol L^{-1} NO_3^- nutrient solution contained 2.5 mmol L^{-1} $Ca(NO_3)_2$, 0.5 mmol L^{-1} $CaCl_2$, 2 mmol L^{-1} K_2SO_4, 2 mmol L^{-1} $MgSO_4$, and 1 mmol L^{-1} KH_2PO_4; and the 10 mmol L^{-1} NO_3^- nutrient solution contained 3 mmol L^{-1} $Ca(NO_3)_2$, 4 mmol L^{-1} KNO_3, 2 mmol L^{-1} $MgSO_4$, and 1 mmol L^{-1} KH_2PO_4. All the solutions contained the same amounts of iron and other micronutrients as the pre-culture nutrient solution. The pH of the solutions was adjusted to 6.5 ± 0.1 with KOH and H_2SO_4. Salinity treatments were introduced gradually from initially 1 to 75 mmol L^{-1} NaCl on day 2 and then at increments of 75 mmol L^{-1} NaCl every 2 d to reduce osmotic shock. Each treatment was replicated three times.

Thirty days after the final salinity concentrations were reached, the plants were removed from the treatment solutions, their roots and shoots were separated, and the fresh weights were determined. Part of the plant tissues were dried for 72 h at 80 °C for determination of dry weights. The rest fresh plant samples were frozen in liquid nitrogen. After washed with distilled water three times, the roots were placed in a 10 g L^{-1} crystal violet solution at 50 °C for 5 min. An abundant volume of dye solution was used and refreshed for staining of each sample. Stained roots were gently rinsed for at least 3 min under running water, carefully spread in a thin layer of water on a transparent tray, and scanned with

an Epson Perfection Photo scanner 1650 (Epson America Inc., USA). The images scanned were saved for analysis of total root length using Rootedge 2.3b software (Himmelbauer *et al.*, 2004).

The length of the primary roots was measured with a ruler to the closest 1 mm. Lateral root length was the difference between total root length and primary root length, and the ratio of the primary root length to the total root length was calculated. All lateral roots including first- and second-order lateral roots that were 5 mm long were counted. Mean length of lateral roots was calculated by dividing total root length by total number of lateral roots.

The frozen plant samples were extracted in boiling distilled water and the extract was filtered. NO_3^- in the filtered extract was determined by a colorimetric method with a UV-120-02 spectrophotometer (Shimadzu, Kyoto, Japan), and Cl^- by titration with 0.03 mmol L^{-1} $AgNO_3$, with 50 g L^{-1} K_2CrO_4 as an indicator. After being ground through a 1-mm screen, the dry plant samples (0.5 g each) were digested in a mixture of nitric, perchloric, and hydrochloric acids (3:2:1) in a Gerhardt digestion block. Na^+ in the digested solution was analyzed by atomic absorption spectrophotometry (Thermo Solaar M, Thermo Electron, USA), and total nitrogen was determined by Kjeldahl method.

Nitrate reductase (NR) activity was measured according to Plaut (1974), with minor modifications. Approximately 0.5 g frozen sample was homogenated in 4 mL of 0.1 mol L^{-1} K-phosphate buffer (pH 7.5) containing 1 mmol L^{-1} EDTA and 1 mmol L^{-1} cysteine. The homogenate was centrifuged for 10 min at 15 000 × g, and the supernatant was used for enzyme assay in a mixture containing 30 μmol L^{-1} K-phosphate buffer (pH 7.5), 0.5 μmol L^{-1} nicotinamide adenine dinucleotide (NADH), 20 μmol L^{-1} KNO_3, and 0.1 mL of the supernatant. The mixture was incubated at 30 °C for 15 min and the reaction was terminated by adding consecutively 1 mL of 10 g L^{-1} sulphanilamide dissolved in 2 mol L^{-1} HCl and 1 mL of 0.2 g L^{-1} *N*-(1-naphthyl)-ethylenediamine. Absorbance was read at 540 nm after 15–20 min.

Statistical analysis was performed with SAS software (version 6.12, SAS Institute Inc., Cary, USA). All data were subject to a two-way analysis of variance (ANOVA) and the means were separated by least significant difference (LSD) test at the 5% level.

RESULTS

Effects of salinity and NO_3^- on plant growth

The shoot and root dry weights progressively increased with both increasing salinity and NO_3^- levels (Fig. 1, Table I). Increasing NaCl levels from 1 to 300 mmol L^{-1} markedly increased shoot and root growth, but no further significant increase occurred at 450 mmol L^{-1} NaCl (Fig. 1a and b). The seedlings treated with 450 mmol L^{-1} NaCl and 0.05 mmol L^{-1} NO_3^- died at the end of the experiment. Shoot growth was unaffected by increasing NO_3^- levels from 0.05 to 5 mmol L^{-1}, but it significantly increased with further increasing NO_3^- levels up to 10 mmol L^{-1} (Fig. 1a). However, root growth progressively increased as NO_3^- was increased from 0.05 to 10 mmol L^{-1} (Fig. 1b).

Increasing salinity led to a significant decrease in root:shoot ratio at various NO_3^- levels because there was a significantly greater allocation of resources to the shoots (Table I, Fig. 1c). Conversely, an increase in NO_3^- caused a significant increase in root:shoot ratio except at 1 mmol L^{-1} NaCl because more resources were allocated to the roots. Interactive effects of salinity and NO_3^- on the root dry weight and the root:shoot ratio were significant (Table I).

Effects of salinity and NO_3^- on root morphology

Salinity and NO_3^- had significant effects on the lateral root length, number of lateral roots, and the mean length of lateral roots (Table I, Fig. 2). Lateral root length was significantly higher at 300 mmol L^{-1} NaCl with 5 and 10 mmol L^{-1} NO_3^-. However, the length of lateral roots was substantially reduced at 300 mmol L^{-1} NaCl and 0.05 mmol L^{-1} NO_3^-. There was a significant increase in lateral root length as NO_3^- increased, especially at 10 mmol L^{-1} NO_3^- (Fig. 2b). A similar trend was found for

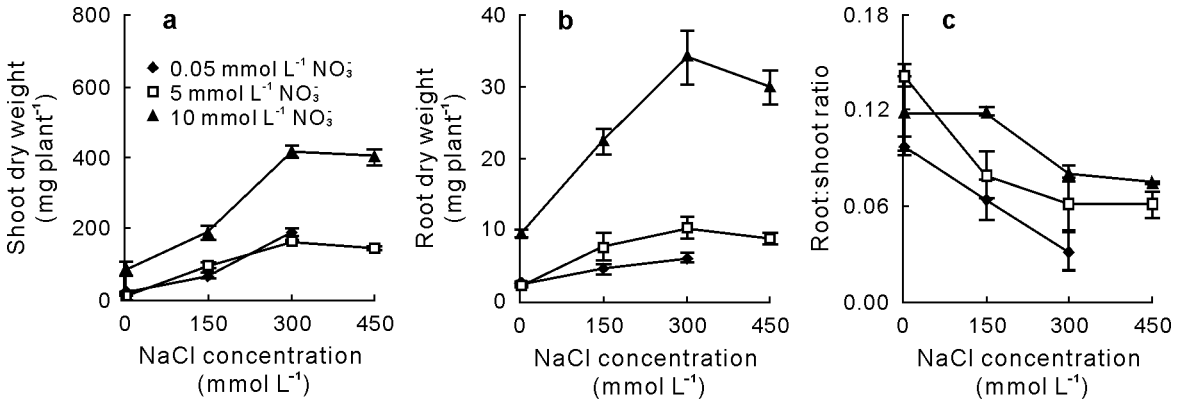

Fig. 1 Shoot dry weights (a), root dry weights (b), and root:shoot ratios (c) of *Suaeda physophora* in response to 1, 150, 300, and 450 mmol L^{-1} NaCl and 0.05, 5, and 10 mmol L^{-1} NO_3^- treatments for 30 d. Vertical bars represent standard errors ($n = 3$).

TABLE I

Results (F values) of two-way analysis of variance for biomass production and allocation and root morphology of *Suaeda physophora* with NaCl and NO_3^- treatments for 30 d ($n = 3$)

Parameter	NO_3^-	Salinity	NO_3^- × salinity
Shoot dry weight	54.25***	48.50***	1.76
Root dry weight	99.03***	27.38***	3.71*
Root:shoot ratio	7.49**	16.54***	2.98*
Primary root length	3.15	0.34	1.60
Lateral root length	137.58***	54.17***	1.90
Number of lateral roots	14.19***	24.25***	0.00
Mean length of lateral roots	66.80***	8.71**	0.45

*, **, ***Significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

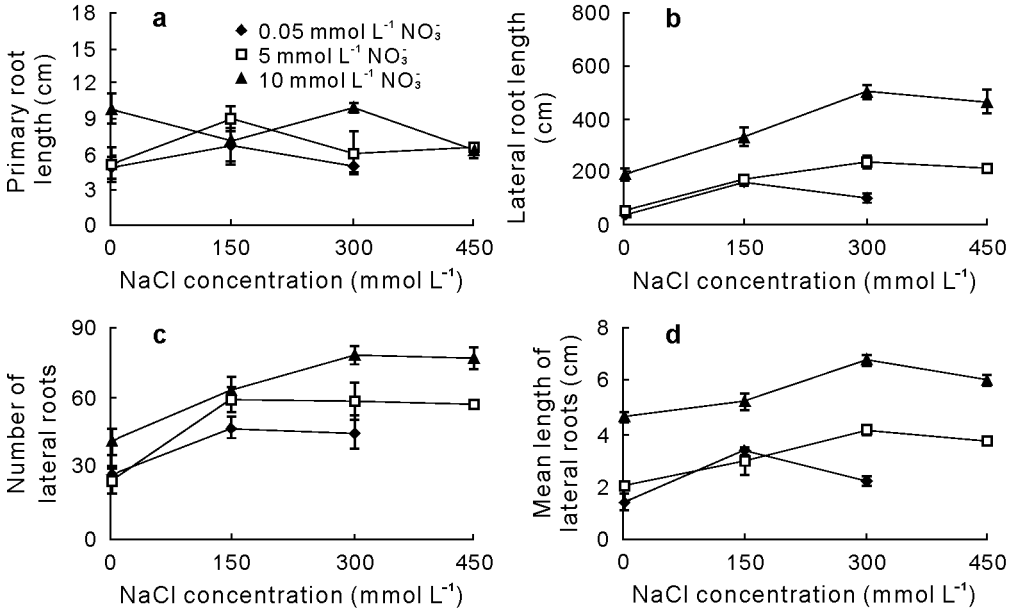

Fig. 2 Primary root length (a), total lateral root length (b), number of lateral roots (c), and mean length of lateral roots (d) of *Suaeda physophora* in response to 1, 150, 300, and 450 mmol L^{-1} NaCl and 0.05, 5, and 10 mmol L^{-1} NO_3^- treatments for 30 d. Vertical bars represent standard errors ($n = 3$).

the mean length of lateral roots (Fig. 2d). Number of lateral roots for plants at 0.05 and 5 mmol L^{-1} NO_3^- increased with increasing NaCl levels up to 150 mmol L^{-1}, but then did not increase further at higher NaCl concentrations. Number of lateral roots at 10 mmol L^{-1} NO_3^- increased with increasing NaCl level up to 300 mmol L^{-1} (Fig. 2c). Salinity, NO_3^-, and their interaction had no significant effect on the primary root length (Table I, Fig. 2a). The primary root represented about 1.4%–11.8% of the total root length.

The effects of salinity and NO_3^- on root distribution were shown in Fig. 3. The depth of the root distribution increased with increasing NO_3^- levels, and the root system in the range of 4–12 cm was larger at 10 mmol L^{-1} NO_3^-. The response of the root distribution to salinity was inconsistent. At 0.05 mmol L^{-1} NO_3^-, the deepest root distribution was observed at 150 mmol L^{-1} NaCl. However, when NO_3^- was increased up to 10 mmol L^{-1}, the deepest roots occurred at 300 mmol L^{-1} NaCl.

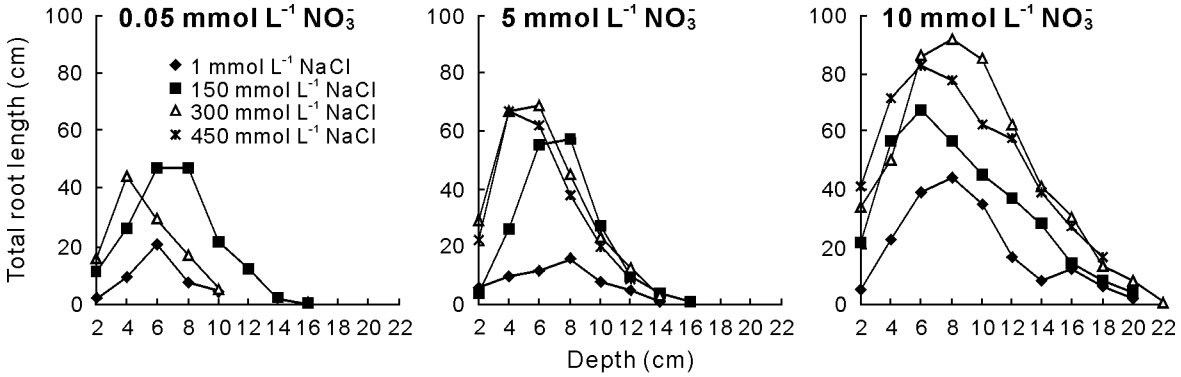

Fig. 3 Total root lengths of *Suaeda physophora* at different depths of the culture solutions in response to 1, 150, 300, and 450 mmol L^{-1} NaCl and 0.05, 5, and 10 mmol L^{-1} NO_3^- treatments for 30 d.

Effects of salinity and NO_3^- on tissue NO_3^- and total nitrogen and NR

The concentrations of NO_3^- and total nitrogen and the NR activities in both roots and shoots except the shoots at 10 mmol L^{-1} NO_3^- significantly decreased with increasing salinity and decreasing NO_3^- supply. Decreased activities of NR with increasing salinity under lower NO_3^- levels were accompanied by decreased tissue concentrations of NO_3^- and total nitrogen (Table II, Fig. 4). At each salinity level, the concentrations of NO_3^- and total nitrogen and the NR activities in the shoots and roots were the greatest at 10 mmol L^{-1} NO_3^-. Salinity and NO_3^- interaction effects were significant on the nitrogen concentration and NR activity in both shoots and roots (Table II).

TABLE II

Results (F values) of two-way analysis of variance for NO_3^-, Na^+, Cl^-, and total nitrogen concentrations and nitrate reductase activity in the shoots and roots of *Suaeda physophora* with NaCl and NO_3^- treatments for 30 d ($n = 3$)

Parameter	NO_3^-		Salinity		NO_3^- × salinity	
	Shoot	Root	Shoot	Root	Shoot	Root
NO_3^-	440.33***	59.19***	16.62**	19.75***	9.69***	4.74**
Total nitrogen	72.57***	78.62***	11.11***	45.58***	10.06***	13.31***
Nitrate reductase activity	25.31***	69.11***	12.30***	64.76***	8.30***	8.41***
Na^+	47.95***	14.36***	183.78***	145.64***	0.00	0.00
Cl^-	141.97***	4.44*	159.14***	159.78***	26.62***	6.67***

*, **, ***Significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Effects of salinity and NO_3^- on tissue concentrations of Na^+ and Cl^-

Increasing salinity led to significant increases in both Na^+ and Cl^- concentrations in the plants

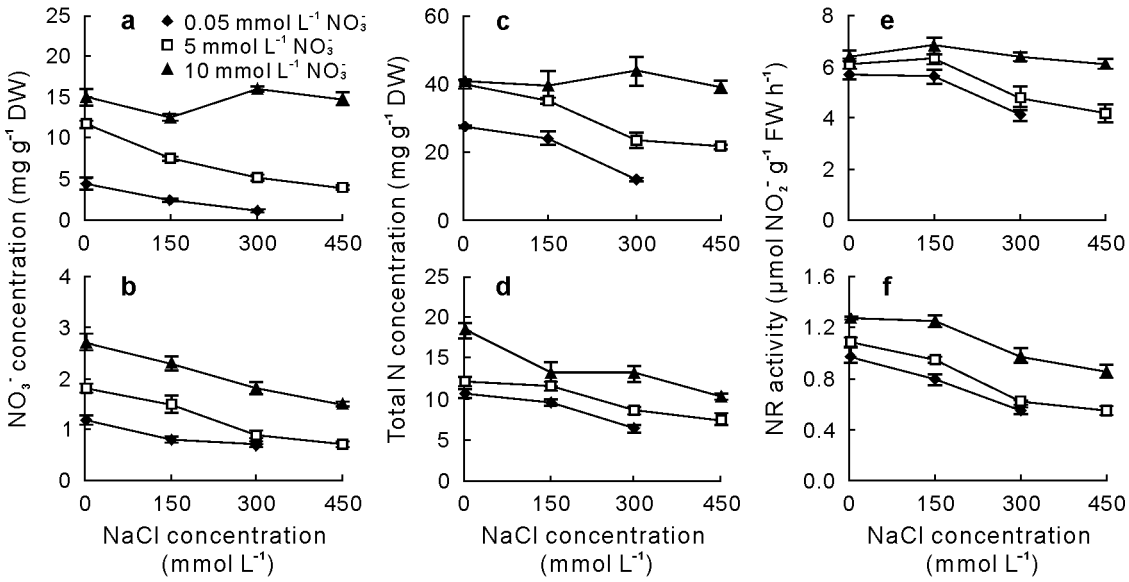

Fig. 4 Concentrations of NO_3^- (a and b), total nitrogen (c and d) and NO_3^- reductase (NR) activities (e and f) in the shoots and roots of *Suaeda physophora* in response to 1, 150, 300, and 450 mmol L^{-1} NaCl and 0.05, 5, and 10 mmol L^{-1} NO_3^- treatments for 30 d. Vertical bars represent standard errors ($n = 3$). DW = dry weight; FW = fresh weight.

(Table II, Fig. 5). The concentration of Na^+ in the shoots increased with increasing NO_3^- supply (Table II, Fig. 5a). However, Cl^- concentrations in both roots and shoot were reduced at 5 and 10 mmol L^{-1} NO_3^- (Table II, Fig. 5c and d). Interactive effects of salinity and NO_3^- on the Cl^- concentrations in the plants were significant (Table II).

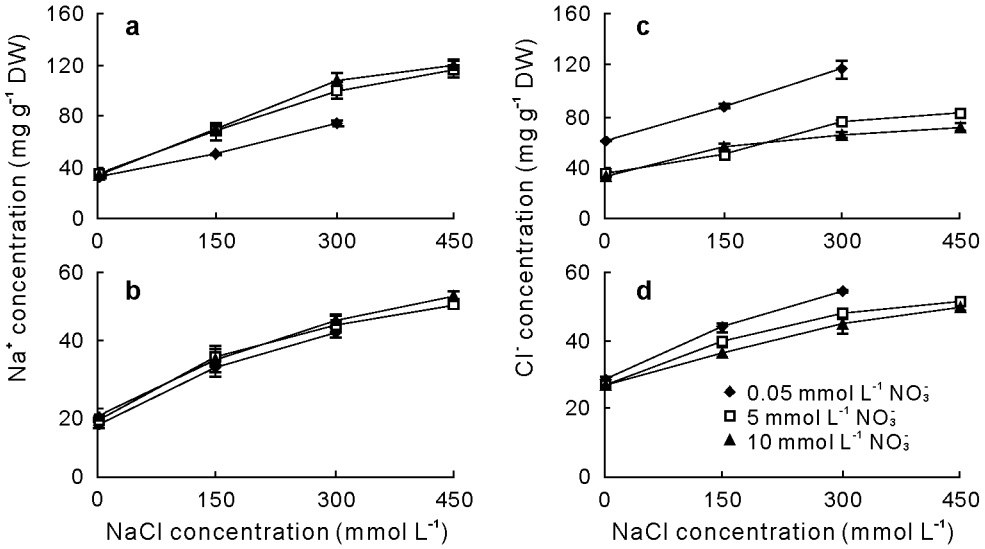

Fig. 5 Concentrations of Na^+ and Cl^- in the shoots (a and c) and roots (b and d) of *Suaeda physophora* in response to 1, 150, 300, and 450 mmol L^{-1} NaCl and 0.05, 5, and 10 mmol L^{-1} NO_3^- treatments for 30 d. Vertical bars represent standard errors ($n = 3$). DW = dry weight.

DISCUSSION

Shoot and root growth

This study revealed the importance of salinity and NO_3^- and their interaction to growth, root mor-

phology, and nitrogen uptake of *S. physophora*. There was a positive shoot growth response to NO_3^- added at 10 mmol L^{-1} under high NaCl salinity ($P < 0.001$) and lower concentrations of NO_3^- had no effect on the shoot growth over a range of external NaCl levels, indicating that *S. physophora* had a high demand for NO_3^- for its shoot growth. This is consistent with the findings of Naidoo (1987), who found that *Avicennia marina* growth at high salinity significantly increased at 14 mg N L^{-1} but not at 0.14 and 1.4 mg N L^{-1} (NH_4Cl).

A similar trend of enhanced growth was found for roots in response to NO_3^- supply under NaCl salinity (Fig. 1b). Similarly, Frechilla *et al.* (2001) found that pea maintained a better root growth under salinity in NO_3^--fed plants. Moreover, root growth of *S. physophora* was more sensitive to NO_3^- addition than shoot growth, which resulted in an increase in the root:shoot ratio (Fig. 1c). These results contrasted with the classical responses to nitrogen supply of most non-halophytes, for which high nitrogen typically reduces root growth relative to shoot growth, and leads to a decrease in the root:shoot ratio (Gleeson, 1993; Coleman *et al.*, 2004). In this study, growth of *S. physophora* was significantly stimulated by NO_3^- (Fig. 1) ($P < 0.001$). However, nitrogen deficiency in plants with only 0.05 mmol L^{-1} NO_3^- at 450 mmol L^{-1} NaCl appeared to lead to the death of all seedlings. Therefore, adequate NO_3^- application substantially increased the growth of *S. physophora* under saline conditions, presumably because NO_3^- was a severe growth-limiting factor compared with salinity. The growth pattern of higher plants manifests an economic principle which is illustrated by the partitioning of photosynthetically produced biomass between roots and shoots in order to achieve optimal utilization of all available resources (Bloom *et al.*, 1985; Van der Werf *et al.*, 1993). The increase in root:shoot ratio of *S. physophora* under high salinity with adequate NO_3^- supply had the potential to increase the ability of the roots to supply nutrients and water, and hence might present an adaptive advantage.

Root morphological features

Lateral root growth was enhanced by increasing NaCl salinity (Fig. 2b) ($P < 0.001$). The increased root proliferation was the result of increased numbers of lateral roots and a significant stimulation of the elongation rate of lateral roots. This is in contrast with the findings of Rubinigg *et al.* (2004) which showed that the lateral root length of *Plantago maritima* was considerably reduced at 200 mmol L^{-1} NaCl, and the decrease was a consequence of the inhibition of the lateral root primordia rather than a reduced length. These showed that different halophytes varied in their responses of lateral root development under high salinity. The presence of NaCl in the nutrient solution may have affected the induction or activation of the apical meristem of lateral roots (Rubinigg *et al.*, 2004). Moreover, the contribution of Na^+ in the solutions to lateral root growth was much higher than that of Cl^- because the concentration of Na^+ in the shoots significantly increased with NO_3^- addition, while the opposite was true for that of Cl^- (Fig. 5). Species in the genus *Suaeda* are salt-accumulating plants; substantial Na^+ concentrations are found in their shoots (Yeo and Flowers, 1980; Wang *et al.*, 2002). Halophyte growth rate appears to be obligately coupled to supply of Na^+ (Yeo and Flowers, 1986). For roots of the non-halophyte cotton, the highest Na^+ concentration was found in the region of the highest localized growth rate (Zhong and Läuchli, 1994). This may suggest that Na^+ accumulation was not the main cause of cotton root growth reduction under NaCl stress. Further studies were required to study the mechanism by which Na^+ regulated lateral root development in halophytes.

In our culture conditions, high external NO_3^- concentration positively affected lateral root branching and growth (Fig. 3b) ($P < 0.001$). It is well known that local NO_3^- favors branching of root systems (Drew and Saker, 1975; Linkohr *et al.*, 2002). Such a stimulation of lateral root elongation in *Arabidopsis* appears to be attributable to a signaling effect from NO_3^- itself, rather than to a downstream metabolite (Zhang and Forde, 1998). In contrast, deficiency of nitrogen led to a remarkable decrease in the length of both first- and second-order lateral roots (Drew and Saker, 1975). In the present study, it appeared that NO_3^- and Na^+ produced a similar effect on the lateral roots, but with the promotive effect of NO_3^- on lateral root length being more pronounced than that of salinity (Table I). On the other hand, nutrient

uptake ability of plants not only depends on root length growth but also on vertical root exploration (Voisin *et al.*, 2002). The depth of the root system of *S. physophora* was highest and the extension of the roots was largest at 10 mmol L^{-1} NO_3^- irrespective of the salinity level (Fig. 3). An extensive root system enhances the ability of plants to absorb both water and nutrients.

There were no significant differences in primary root length at any salinity and NO_3^- levels. The fact that the primary roots represented only about 1.4%–11.8% of the total root length suggested that this part of root system played a minor role in nitrogen and water uptake under salinity. This is in agreement with a previous report for *P. maritima* (Rubinigg *et al.*, 2003). It seems to be a general rule in plants that primary root growth is much less sensitive to nutritional effects than the growth of second- or higher-order roots (Forde and Lorenzo, 2001).

NO_3^- and total nitrogen concentrations and NR activity in the shoots and roots

In evaluating the effect of salinity on plant NO_3^- and total nitrogen concentrations it should be stressed that the response was not the same between the shoots and roots. Within each NO_3^- level, raising salinity reduced the concentrations of NO_3^- and total nitrogen and the NR activities in the roots and shoots except the shoots at 10 mmol L^{-1} NO_3^- (Fig. 4). This implied that the well-established NO_3^- uptake and transport systems to the shoots of *S. physophora* were probably unaffected by high Cl^- concentrations in the plant. Torres and Bingham (1973) found that the most NaCl-tolerant cultivars of wheat were those which had high leaf NO_3^- concentrations whether the plants were grown under saline conditions or not. High salt tolerance in *S. physophora* with optimal nitrogen availability may also be partly due to an ability to maintain the high shoot NO_3^- concentrations. NO_3^- can act as an osmoticum, filling vacuoles and driving growth (McIntyre, 1997; Song *et al.*, 2006). The cost of increasing tissue nitrogen concentration is found to be primarily related to an increase in nitrogen allocation to roots (Hilbert, 1990). The large root system of *S. physophora* under salinity with adequate NO_3^- would have been able to ensure high NO_3^- uptake and transport to the shoots. Dalton *et al.* (2000) suggested that plants with larger root growth relative to shoot growth will have a higher ion loading into shoots (Zhang *et al.*, 1999). However, root growth was not only dependent on the prevailing external nutritional supply but also on the nutrient status of the plant as a whole. Further work is needed to clarify the potential contribution of shoot nitrogen demand to root system development of *S. physophora*.

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