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REGULAR ARTICLE

Effects of NO_3^--N on the growth and salinity tolerance of *Tamarix laxa* Willd

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Abstract The influence of NO_3^--N on growth and osmotic adjustment was studied in *Tamarix laxa* Willd., a halophyte with salt glands on its twigs. Seedlings of *T. laxa* Willd. were exposed to 1 mM (control) or 300 mM NaCl, with 0.05, 1 or 10 mM NO_3^--N for 24 days. The relative growth rate of seedlings at 300 mM NaCl was lower than that of control plants at all NO_3^--N levels, but the concentrations of organic N and total N in the twigs did not differ between the two NaCl treatments. Increasing NO_3^- supply under 300 mM NaCl improved the growth of *T. laxa*, indicating that NO_3^- played positive roles in improving salt resistance of the plant. The twigs of *T. laxa* Willd. accumulated mainly inorganic ions, especially Na^+ and Cl^-, to lower osmotic potential (Ψs): the contributions of Na^+ and Cl^- to Ψs were estimated at 31% and 27% respectively, at the highest levels of supply of both NaCl and NO_3^--N. The estimated contribution of NO_3^--N to Ψs was as high as 20% in the twigs in these conditions, indicating that NO_3^- was also involved in osmotic adjustment in the twigs. Furthermore, increases in tissue NO_3^- were accompanied by decreases in tissue Cl^- and proline under 300 mM NaCl. The estimated contribution of proline to Ψs declined as with NO_3^--N supply increased from 1 to 10 mM, while the contributions of nitrate to Ψs were enhanced under 300 mM NaCl. This suggested that higher accumulation of nitrate in the vacuole alleviated the effects of salinity stress on the plant by balancing the osmotic potential. In conclusion, NO_3^--N played both nutritional and osmotic roles in *T. laxa* Willd. in saline conditions.

Keywords *Tamarix laxa* willd · Salt stress · NO_3^--N · Osmoregulation · Proline

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Introduction

Salinity reduces plant growth through osmotic stress, ion toxicity, and consequently nutritional stress (Nublat et al. 2001). Many studies showed that improving of the nutritional status of plants by nitrogen or phosphorus fertilizers may mitigate the negative impacts of increased salinity and promote the

growth of plants (Ebert et al. 2002; Papadopoulos and Rendig 1983). Such a role is believed as an indirect effect of nitrogen on salinity resistance in plants.

Nitrogen (N) shortage is one of the main factors limiting plant growth in many ecosystems, particularly in saline soils (Albassam 2001; Botella et al. 1997). Some saline soils, however, e.g. nitric saline soil (belonging to the Typic Salorthids) in Turpan Basin, Xinjiang, northwestern China, contain nitrate concentration as high as 2–16 g kg^{-1} in 0–30 cm soil layer (Wang et al. 1993; Yang 1999). Several plant species grow in these nitric saline soils, such as *Tamarix* spp., *Phragmites australis* L., *Suaeda pterantha* (Kar.et Kir.) Beg, *Kalidium foliatum* (Pall.) Moq, *Halopeplis pygmaea* (Pall.) Bge, *Saliconia europaea* L., *Halostachys caspica* (Bieb.) C. A. Mey., *Artemisia anethifolia* Web. ex Stechm, *Aeluropus pungens* (M. Bieb.) C. Koch., and *Karelinia caspica* (Pall.) Less (Xi et al. 2006).

Previous research on the relationship between N and salt resistance in plants has mainly focused on the assimilation of N and/or the secondary metabolism of N-containing compounds. For example, increased N supply can promote salt resistance in plants by accumulation of soluble N containing organic compounds (e.g., proline, glycinebetaine and free amino acids) under salinity stress (Dubey and Pessarakli 1995). The possible role of these compatible solutes is in protecting the plants against photoinhibition (Hayashi et al. 1997; Holmström et al. 2000; Yang et al. 2007), reactive oxygen species (Demiral and Türkan 2004; Heidari and Mesri 2008) or osmotic stress in plants growing under salt stress (Hasegawa et al. 2000; Shen et al. 1999; Yancey 2005).

Salinity decreases NO_3^- uptake by roots (Martinez and Cerda 1989), inhibits the activity of nitrate reductase (Campbell 1988), and reduces NO_3^- translocation from roots to shoots (Gouia et al. 1994). On the other hand, salinity stress tends to result in an increase accumulation of proline (Moghaieb et al. 2004; Parida and Das 2005) in non-halophytic plants (Albassam 2001) and also in halophytes (Khan et al. 2000), and the levels of nitrogenous solutes rises as N supply increases, e.g., *Spartina alterniflora* (Colmer et al. 1996).

NO_3^- is the main form of N uptake by angiosperms (Martinoia et al. 1981). When NO_3^- absorption exceeds reduction capacity, the excess NO_3^- will be stored in vacuoles (Blom-Zandstra and Lampe 1983). Some ecological factors, e.g. light intensities (Blom-Zandstra and Lampe 1985) or osmotic stress (Ourry et al. 1992), may suppress the activity of nitrate reductase and result in NO_3^- accumulation in vacuoles. Marschner (1986) had hypothesized that the storage of NO_3^- in vacuoles might play a role in osmotic regulation when plants were growing in osmotic stress. However, previous studies have mainly focused on the nutritional function of NO_3^-, just a few studies have been conducted to assess Marschner's hypothesis by testing the direct contribution of NO_3^- to the salt resistance of plants, and the results varied. For example, Stienstra (1986) showed that NO_3^- did not have a specific function in osmotic adjustment in *Aster tripolium* L., a halophytic plant, grown in a nutrient solution with either a continuous or an intermittent NO_3^- supply. By contrast, Song et al. (2006a) suggested that NO_3^- not only has a nutritional role for growth of the euhalophyte *Suaeda physophora*, but also directly contributes to osmotic adjustment. In order to test Marschner's hypothesis, a recretohalophyte (a halophyte with salt excretion glands), *Tamarix laxa*, was evaluated in hydroponic culture under controlled conditions, with three NO_3^- treatments under low (1 mM) and high (300 mM) NaCl-salinity.

Materials and methods

Plant material

Branches of *T. laxa* Willd. of 0.5±0.1 cm in diameter were collected in November 2006, from Fukang, Xinjiang, China (44°18′ N, 87°55′ E). Plant samples were chopped into 10–12 cm segments, dipped in water for 24 h, sterilized with 0.5% potassium permanganate for 30 min, and then washed with tap water. The basal part of each segment was dipped in 0.1% rooting powder (ABT Rooting Powder®, produced by ABT Research and Development Center of Chinese Academy of Forestry, Beijing, China) for 10 h. The segments were then cultured in quartz sand for rooting and supplied initially with tap water. When new twigs appeared, the plants were cultured with 1/10 strength nutrient solution for 30 d and then with 1/2 strength nutrient solution renewed every 2 days for another 40 days. The nutrient solution composition at full strength was: 0.05 mM $Ca(NO_3)_2$,

2.95 mM $CaCl_2$, 1 mM K_2SO_4, 2 mM $MgSO_4$, 1 mM KH_2PO_4, 90 μM Fe-EDTA, 46 μM H_3BO_3, 9.1 μM $MnCl_2$, 0.32 μM $CuSO_4$, 0.76 μM $ZnSO_4$, 0.56 μM Na_2MoO_4, 1 mM NaCl. The pH was adjusted each day to 6.5±0.1 with KOH or H_2SO_4. The pots were in a greenhouse with a light intensity of 480 μmol m^{-2} s^{-1}, and temperature of 30±3°C in day and 24±3°C in night. The relative humidity was 50–60%. In order to prevent salt accumulation in the quartz sand, relatively large volumes of fresh nutrient solution was used to irrigate the pots to leach out any excess salts every 2 days.

Experimental design

Plants were washed with deionized water, then transferred into 1/2 strength nutrient solution for 7 days. The fresh weight of each seedling was measured, and similar-sized seedlings with fresh weight 5.0±0.2 g were selected before the pretreatment. The experiment was arranged in a completely randomized design with two factors: (1) two salinity levels, 1 mM (control) and 300 mM NaCl (salinity); (2) three nitrogen levels, 0.05, 1 and 10 mM NO_3^--N supplied as $Ca(NO_3)_2$. The extra Ca^{2+} concentration in different treatments was balanced by $CaCl_2$. In order to avoid osmotic shock, 300 mM NaCl was applied gradually by adding 50 mM NaCl per day. The plants were cultured in 2 L porcelain pots. Each pot contained two seedlings. Three seedlings of similar fresh weight per treatment were collected for dry weight (DW) when the final salinity concentrations were reached (as the initial dry weight for calculating relative growth rate). There were six replicates for each treatment, in which three replicates were used for evaluating biomass, and the other three were used for determining physiological parameters. The experiment was terminated 24 days after final salinity concentrations were reached. The twigs and roots were separated, and fresh weight (FW) was recorded. A sub-sample of fresh twigs samples of each plant was frozen in liquid N_2. The remaining plant tissue samples were dried in an oven at 80°C for 72 h and dry weights (DW) were measured. Water content (WC) was calculated as: (FW-DW) / DW. The concentrations of Na^+, K^+ and organic N were measured in oven-dried samples. Osmotic potential, and the concentrations of Cl^-, NO_3^- and proline, were measured in samples frozen in liquid N_2.

Determination of the relative growth rates of plants

Plants were sampled at the beginning of treatments and at the end of the experiment. Relative growth rate (RGR) was calculated using the equation (Botella et al. 1997):

$$RGR = (\log_e W_2 - \log_e W_1)/(t_2 - t_1)$$

Note: W_1 and W_2 represent plant fresh weight at harvest 1 (1 day after the final salinity concentrations were reached) and harvest 2 (at the end of the experiment) respectively, over the harvest interval t_1 to t_2 (1 to 24 days).

Determination of inorganic ions, proline, amino acids, and organic N in plants

Frozen plant material (vegetative branches and roots of *T. laxa*) was extracted with boiling distilled water, and Cl^- and NO_3^- were determined after the solution was filtered. NO_3^- was determined by the colorimetric method (Cataldo et al. 1975) (UV-120-02 Spectrophotometer, Shimadzu, Kyoto, Japan), and Cl^- was determined by 0.03 mM $AgNO_3$ titration method, with 5% K_2CrO_4 as indicator. Frozen plant tissue was also ground in 10% acetic acid, and the ninhydrin colorimetric method was used for the determination of amino acids (Moore and Stein 1954), or the concentration of proline (Troll and Lindsley 1955). For cation determinations, about 15-mg dry sample was put in a muffle stove to be ashed. The ash was dissolved in 0.1 ml of concentrated nitric acid and then diluted to a volume of 20 ml with deionized water. The concentrations of Na^+ and K^+ were determined by flame photometry (Model 2655-00 Digital Flame Analyzer, Cole-Parmer Instrument Company, Chicago, USA). Dry plant samples were ground, and analyzed for organic N using the Kjeldahl method (Shi 1994).

Determination of osmotic potential (Ψs)

The frozen plant tissues were put into a syringe to thaw. The liquid squeezed from the plant tissues was analysed using a freezing point osmometer (Fiske 210; Advanced Instruments Inc., Norwood, MA, USA) to measure the *ic* value (the value reading from the instrument). The tissue osmotic potential of solutes was calculated as $\Psi s = -icRT$, where i is ionization constant of the solute, c is the molar concentration of the solute, R is the

universal gas constant and T is the temperature in degrees Kelvin (Song et al. 2006a).

Vacuoles account for most of the volume of the twigs, particularly in recretohalophyte plants which have large water storage cells (Bosabalidis and Thomson 1985; Thomson 1975). For the present analyses, the volume of the cytoplasm is assumed to occupy 10% of cells. Therefore we calculated the average concentration of each inorganic ion in vacuole or organic solute in cytoplasm by the content of each individual osmolyte and the tissue water content with the volume ratio of vacuole vs cytoplasm 9:1. The osmotic potential (Ψ) of each individual osmolyte, such as Na^+, K^+, Cl^-, NO_3^-, or proline, was calculated by $\Psi = -nRT/V$, where n is the number of solute molecules, R is the universal gas constant, T is temperature in °K, and V is the volume in liter. Osmotic coefficients of the solutes in tissue water were assumed to equal 1 (Song et al. 2006a).

The estimated contributions of each individual solute (C_{es}) to the tissue osmotic potential was calculated using the equation: $C_{es}(\%) = \Psi/\Psi s \times 100$.

Statistical analysis

All data were subjected to a two-way ANOVA using the SAS™ software (SAS Institute Inc. 1989). Treatment means were compared by least significant differences (LSD) at $P=0.05$.

Results

Effect of NO_3^--N on the growth of *T. laxa* under salinity stress

No differences were observed in the growth of *T. laxa* twigs growing at 1 and 10 mM NO_3^--N at either NaCl level ($P<0.05$) (Table 1). However, twig fresh weights were much lower when grown at 0.05 mM NO_3^--N under either NaCl level (Table 1). There were no significant differences in root fresh weights among the three levels of NO_3^--N under either NaCl levels (Table 1). Root / twig ratios were significantly higher in plants grown at 0.05 mM NO_3^--N, compared to values for plants grown at 1 and 10 mM NO_3^--N under either NaCl levels. These results indicated that increased NO_3^- supply improved twig growth to some degree regardless of salinity level.

The average twig growth of plants at 300 mM NaCl was reduced compared to the low salt treatment, at all NO_3^--N levels (Table 1). Twig growth at 0.05 mM NO_3 was similar at both 1 and 300 NaCl but differed between the two salt levels at 1 and 10 mM nitrate. Twig fresh weight under 1 mM NaCl at 1 and 10 mM NO_3^--N was 121% and 133% higher respectively compared to values obtained at 300 mM NaCl, whereas average root fresh weight

Table 1 Effects of NaCl and NO_3^--N on the growth of root and twig, root/twig ratio and relative growth rate of *T. laxa* seedlings. Plants were treated with 1 or 300 mM NaCl and 0.05, 1 or 10 mM NO_3^--N for 24 days

NaCl (mM)	NO_3^--N (mM)	increments of f. wt (g/plant)		Root / Twig	RGR ($g \cdot g^{-1} \cdot day^{-1}$)
		Twig	Root		
1 mM	0.05	3.90 b[a]	1.39 a	0.36 a	0.083 b
	1	11.64 a	2.16 a	0.19 b	0.111 a
	10	13.07 a	2.49 a	0.19 b	0.103 a
Mean[b]		9.54 A	2.01 A	0.24 B	0.097 A
300 mM	0.05	3.08 b	1.08 a	0.35 a	0.051 b
	1	5.27 a	1.60 a	0.30 b	0.094 a
	10	5.60 a	1.58 a	0.28 b	0.083 a
Mean		4.65 B	1.75 A	0.31 A	0.080 B

All data are means of 3 replications

Increments of f. wt (g/plant) were calculated by fresh weight at the end of the experiment minus fresh weight at the beginning of treatments

[a] Values marked with different letter represented significant difference at $P=0.05$ level across all NO_3^--N levels at a given NaCl level

[b] Means value for each NaCl level. Means values marked with different capital letter indicate significant differences at $P=0.05$ level between NaCl levels

under 300 mM NaCl did not differ from that at 1 mM NaCl (Table 1). Compared with 1 mM NaCl, the average root/twig ratio was increased under the 300 mM NaCl treatment, but the average relative growth rate (RGR) decreased (Table 1). These results indicated that 1 mM NO_3^--N was enough to support maximal twig growth of *T. laxa* under high salinity.

The concentrations of NO_3^--N, Cl^-, Na^+, K^+ in twigs and roots of *T. laxa*

NO_3^- concentrations in twigs of *T. laxa* were enhanced with increasing NO_3^--N supply under 1 or 300 mM NaCl (Fig. 1a). Similar trends of NO_3^- concentrations were found in roots (Fig. 1b). Compared with plants growing at 1 mM NaCl, 300 mM NaCl reduced the NO_3^- concentration in twigs of plants growing at both 1 mM NO_3^--N and 10 mM NO_3^--N ($P<0.05$), but did not affect the NO_3^- concentration in roots (Fig. 1a, b).

The concentration of Cl^- in the twigs decreased with increasing NO_3^--N under both NaCl level except at 10 mM NO_3^--N and 300 mM NaCl treatment where Cl^- concentration showed no significant difference comparing to 1 mM NO_3^--N and 300 mM NaCl treatment (Fig. 1c). In roots, the concentrations of Cl^- were significantly higher at 300 mM NaCl than those in 1 mM NaCl at all NO_3^--N levels (Fig. 1d).

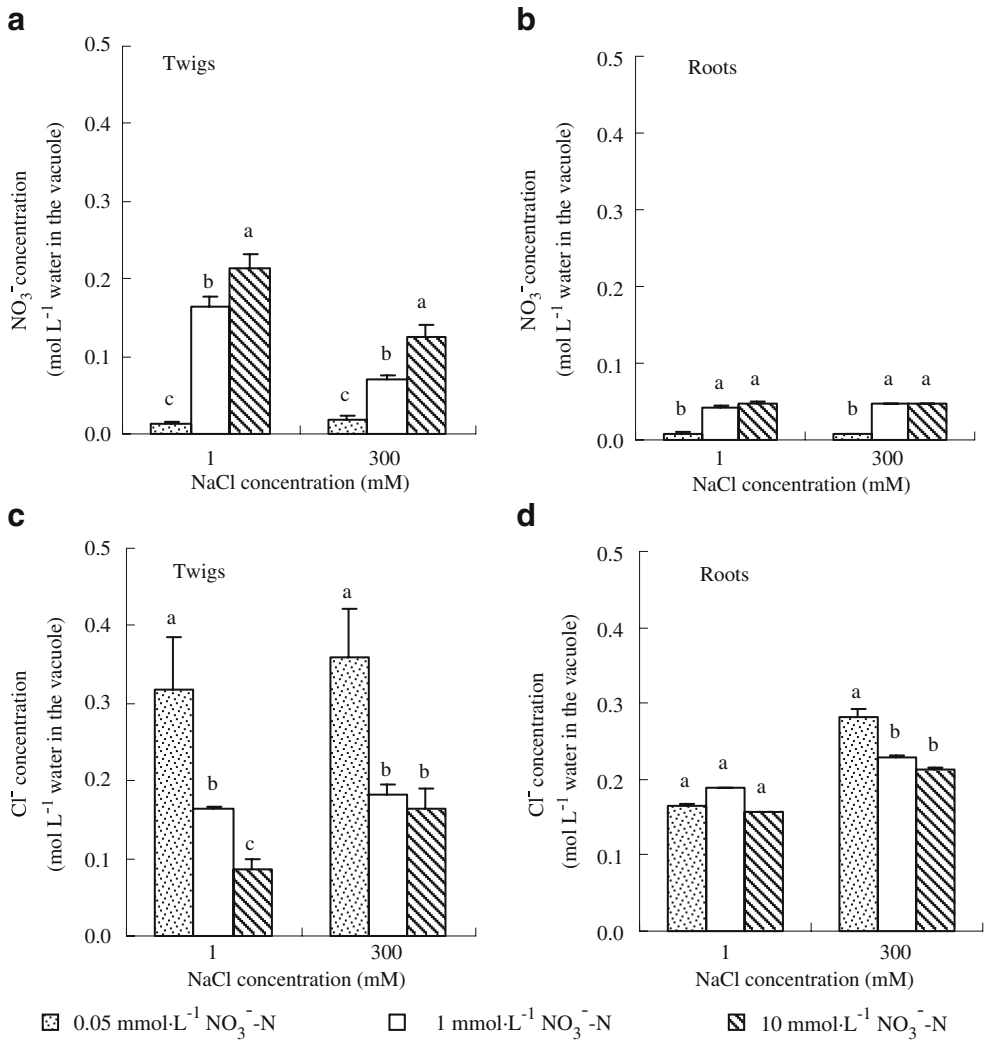

Fig. 1 NO_3^- (**a**, **b**) and Cl^- (**c**, **d**) concentrations in the vacuole in twigs and in roots of *T. laxa*, which were treated with 1 or 300 mM NaCl and 0.05, 1 or 10 mM NO_3^--N for 24 days

Comparing to low NO_3^--N supply, medium and high NO_3^--N levels reduced Cl^- concentration in root at 300 mM NaCl, while no significant differences were observed between the three NO_3^--N levels at 1 mM NaCl (Fig. 1d). These results implied that higher NO_3^--N supply reduced Cl^- uptake.

Salinity increased the concentration of Na^+ in both twigs and roots (Fig. 2a, b). At 1 mM NaCl, the concentration of Na^+ in twigs and in roots did not differ with increasing NO_3^--N supply. At 300 mM NaCl, the Na^+ concentration was higher at 0.05 mM NO_3^--N than at 1 or 10 mM NO_3^--N (Fig. 2a, b). These results indicated that supply of higher NO_3^--N levels might have reduced the concentrations of Na^+ in both twigs and roots under higher salinity.

K^+ concentration both in twigs and in roots were not significantly different among NO_3^--N treatments under either NaCl level (Fig. 2c, d).

Contents of total N, organic N, and NO_3^--N in twigs and roots of *T. laxa* with increasing supply of NO_3^--N at 1 and 300 mM NaCl

The contents of total N (N_{total}), organic N (N_{org}) and NO_3^--N in both twigs and roots were all enhanced with increasing supply of NO_3^--N under both NaCl levels compared to the values at 0.05 mM NO_3^--N

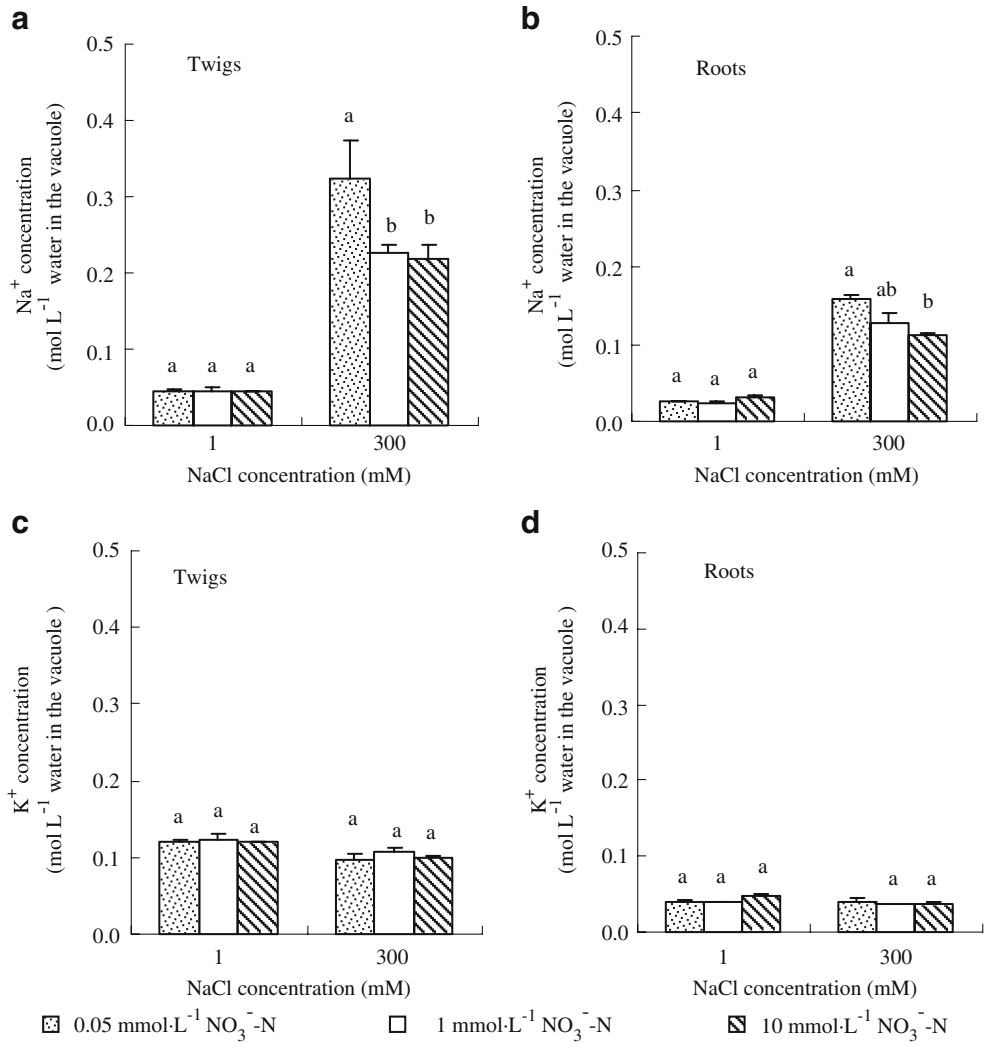

Fig. 2 Effect of NO_3^--N supply on the concentrations of Na^+ (**a**, **b**), K^+ (**c**, **d**) in the vacuole in twigs and roots of *T. laxa* which were treated with 1 or 300 mM NaCl, with 0.05, 1 or 10 mM NO_3^--N for 24 days

supply (Table 2). In the high salinity treatment (300 mN NaCl), the contents of either total N (N_{total}), or organic N (N_{org}) in twigs were similar between treatments of 1 and 10 mM NO_3^--N, whereas NO_3^--N contents in twigs were significantly different across all three NO_3^--N levels at this salinity

The concentrations of total N (N_{total}) and organic N in twigs were enhanced in the 300 mM NaCl treatment compared with those at 1 mM NaCl, whereas NO_3^- concentrations were reduced ($P<0.05$). Salinity had no significant effect on the concentrations of total N (N_{total}) and NO_3^--N in roots.

Effect of NO_3^--N supply on the osmotic potential (Ψs) and the estimated contribution (C_{es}) of Na^+, K^+, Cl^- and NO_3^- to Ψs in twigs of *T. laxa*

The NO_3^--N supply had no effect on the Ψs in twigs of *T. laxa*, while salinity significantly reduced the Ψs (Table 3). The estimated contribution of Na^+ to Ψs (C_{Na}) decreased with increasing NO_3^--N supply at 300 mM NaCl (Table 3). However, there were no significant differences in the C_{Na} among the three NO_3^--N levels at 1 mM NaCl. C_{Na} increased with salinity, from about 7.23% at 1 mM NaCl up to 35.78% at 300 mM NaCl (Table 3).

The estimated contribution of Cl^- to Ψs (C_{Cl}) declined with the increasing NO_3^--N at both NaCl level. The decrease in contribution of Cl^- with increasing NO_3^--N was more marked at 1 mM NaCl than at 300 mM although the interaction was not statistically significant (Table 3).

The estimated contribution of K^+ to Ψs (C_K) decreased with increasing NO_3^--N supply at low salinity, but there were no significant differences among the different NO_3^--N levels under 300 mM NaCl (Table 3). C_K decreased with increased NaCl supply.

The estimated contribution of NO_3^- to Ψs (C NO_3^-) increased with the increase of NO_3^--N at both 1 mM and 300 mM NaCl, whereas it was lower at high salinity at any given NO_3^- level (Table 3).

Effect of NO_3^--N supply on the concentration and the estimated contribution of proline to Ψs in twigs of *T. laxa*

Based on the assumption that proline was restricted to the cytosol, and that that accounted for 10% of the total cell volume, there was a significant increase in

Table 2 The effects of NO_3^--N on the contents of total N, organic N and NO_3^--N in twigs and roots of *T. laxa* which were treated with 1 or 300 mM NaCl and 0.05, 1 or 10 mM NO_3^--N for 24 days

NaCl	NO_3^--N	Ntotal (mg·g^{-1} DW)		Norg (mg·g^{-1} DW)		N NO_3^- (mg·g^{-1} DW)	
(mM)	(mM)	Twig	Root	Twig	Root	Twig	Root
1	0.05	20.88 c[d]	13.60b	20.34b	12.57b	0.54c	0.91b
	1	37.80b	22.87a	32.11a	17.98a	5.69b	4.37a
	10	43.50a	25.94a	34.72a	20.59a	8.79a	3.74a
	Mean[e]	34.06 B[f]	20.80A	29.06B	17.05B	5.01A	3.01A
300	0.05	26.51b	16.26b	25.60b	15.38c	1.03c	0.87b
	1	39.80a	25.25a	35.42a	19.82a	4.9b	5.44a
	10	37.83a	22.75a	34.09a	17.48b	5.34a	5.27a
	Mean	34.71A	21.42A	31.70A	17.56A	3.76B	3.86A
Analysis of Variance (F Values)							
Salinity (S)		8.84[b]	1.27 [NS]	6.62[a]	1.47[NS]	10.96[b]	0.77 [NS]
NO_3^--N level (N)		15.85[c]	78.81[c]	67.05[c]	25.02[c]	15.85[c]	95.09[c]
S × N		3.69 [NS]	8.61[NS]	2.94[NS]	8.59[NS]	8.71[b]	4.03[NS]

[a] denotes significant difference at $P=0.05$, [b] denotes significant difference at $P=0.01$, [c] denotes significant difference at $P=0.001$, NS denotes no significant difference. Data represent F values

[d] Within each column, values with different letter are significantly different at $P=0.05$ level across all NO_3^--N levels

[e] Mean value for each NaCl level

[f] Mean values with different capital letter are significantly different at $P=0.05$ level between NaCl levels

Table 3 The effects of NO_3^--N on the osmotic potential (Ψs), water content (WC), the estimated contribution of Na^+ (CNa^+), K^+ (CK^+), Cl^- (CCl^-) and NO_3^- (C NO_3^-) to osmotic potential in twigs of *T. laxa* which were treated with 1 or 300 mM NaCl and 0.05, 1 or 10 mM NO_3^--N for 24 days

NaCl (mM)	NO_3^--N (mM)	Ψs (MPa)	WC (ml·g^{-1} DW)	CNa^+ (%)	CCl^- (%)	CK^+ (%)	C NO_3^- (%)
1	0.05	−1.30a[d]	3.03c	7.52a	54.2a	20.72a	2.4b
	1	−1.25a	3.81b	7.85a	28.94b	21.77a	29.2a
	10	−1.61a	4.14a	6.32a	11.89c	16.47b	29.47a
	Mean[e]	−1.39A[f]	3.66A	7.23B	24.00B	19.46A	15.44A
300	0.05	−1.71a	3.87a	42.16a	46.61a	12.57a	2.29c
	1	−1.48a	3.96a	34.03b	27.4b	15.99a	10.72b
	10	−1.55a	3.90a	31.14b	27.08b	14.12a	19.93a
	Mean	−1.58B	3.91A	35.78A	33.7A	14.22B	10.31B
Analysis of Variance (F Values)							
Salinity (S)		0.16 ^{NS}	10.67^a	83.77^c	56.07^c	41.03^c	35.53^b
NO_3^--N level (N)		0.68 ^{NS}	17.2^c	1.37^{NS}	4.20^a	6.08^a	61.01^c
S × N		0.03 ^{NS}	14.51^b	0.97^{NS}	0.7 ^{NS}	3.96^{NS}	10.04^c

[a] denotes significant difference at P=0.05, [b] denotes significant difference at P=0.01, [c] denotes significant difference at P=0.001, NS denotes not significant difference. Data represent F values

[d] Within each column, values with different letter are significantly different at P=-0.05 level across all NO_3^--N levels

[e] Mean value for each NaCl level

[f] Mean values with different capital letter are significantly different at P=0.05 level between NaCl levels

proline concentration at 300 mM NaCl compared to the low salinity treatment. Concentrations of proline rose with increasing NO_3^--N supply at 1 mM NaCl; However, at 300 mM NaCl, concentrations of proline were maximum at 1 mM NO_3^--N supply, which indicated that 1 mM NO_3^--N supply was enough for structural growth; however, as the level of NO_3^--N supply increased to 10 mM the concentration of proline decreased as more NO_3^- were being stored in the vacuoles. The concentration of proline was the lowest at 0.05 mM NO_3^--N which implied that the proline synthesis was limited because of nitrogen deficiency (Fig. 3a).

The estimated contribution of amino acids to Ψs (C_{pro}) increased with increasing NO_3^--N at 1 mM NaCl. At 300 mM NaCl, it increased significantly when the NO_3^--N supply was increased from 0.05 to 1 mM, but then dropped at 10 mM NO_3^--N (Fig. 3b). In general, the estimated contribution of proline to Ψs (C_{pro}) was considerably lower at both salinities and all three NO_3^--N levels compared with the inorganic solutes described above: the maximum contribution of proline accounted for only 3.6% of Ψs.

Discussion

Nitrogen is an essential nutrient for higher plants. Salinity may suppress the uptake and assimilation of nitrate in plants, which results in nutritional disorder and growth inhibition (Dluzniewska et al. 2007; Marschner 1986). In the present study, high salinity reduced twig growth and the relative growth rate (RGR) of *T. laxa* plants (Table 1). However, the average total nitrogen or organic nitrogen contents in plants grown under 300 mM NaCl were higher than those grown under 1 mM NaCl, while the average NO_3^- concentration was lower at high NaCl (Table 2). Such results indicated that the uptake and assimilation of NO_3^- in *T. laxa* were not suppressed by high salinity. Increasing the nitrogen supply under either level of NaCl supply improved the RGR of *T. laxa* (Table 1), which can be partly attributed to the nutritional role of N as many other researchers have concluded (Irshad et al. 2008; Leidi et al. 1992).

In order to lower water potential, halophytes accumulate large amounts of inorganic ions in the vacuole and synthesize a relatively small amount of low molecular weight organic compounds to balance

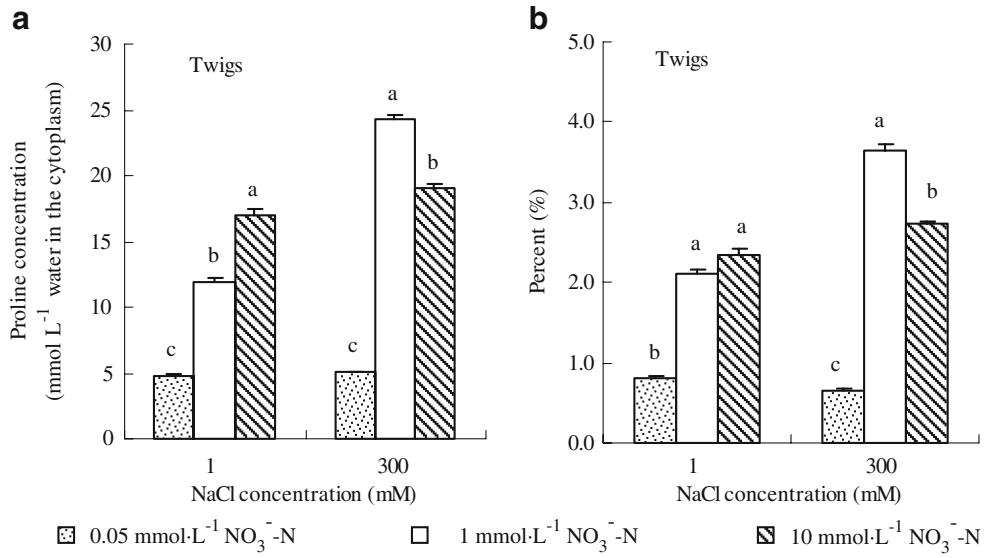

Fig. 3 Proline concentration (**a**) and the estimated contribution of proline to *Ψs* (**b**) in the cytoplasm in twigs of *T. laxa* which were treated with 1 or 300 mM NaCl and 0.05, 1 or 10 mM NO_3^--N for 24 days

the osmotic pressure in the cytoplasm (Hasegawa et al. 2000; Zhao et al. 2003). Song et al. (2006b) demonstrated that euhalophyte *Suaeda physophora* is able to compartmentalize inorganic ions, especially Na^+ in the vacuole, and synthesize a relatively small amount of organic solutes to balance the osmotic pressure in the cytoplasm. In the present study, *T. laxa* accumulated Na^+ and Cl^- in twigs under high salinity (Figs. 1, 2), which would lower osmotic potential in the twigs (Table 3). However, it is difficult to directly test the real concentration of the solutes: it was assumed that inorganic solutes were mainly distributed in vacuole and that organic solutes entirely accumulated in cytoplasm. In the present study, we estimated the concentrations of inorganic or organic solutes by tissue water volume and the general ratio of vacuole to cytoplasm. The distribution was based on the assumption, justified by anatomical studies, that the vacuole accounted for 90% and the cytoplasm and organelles for 10% of the overall cell volume (Di Martino et al. 2003). Therefore the relative estimated-contribution of the inorganic or organic solutes to osmotic potential can be quantified (Silveira et al. 2009).

Although Marschner (1986) had hypothesized that NO_3^- stored in vacuoles might play a role in osmotic regulation when plants were growing in osmotic stress, whether or not NO_3^- plays this role and directly contributes to salt resistance of plants is still poorly understood. As noted in the introduction, the answer may depend on the species being considered. Our present study showed that NO_3^--N supply significantly enhanced the contribution of NO_3^- to osmotic potential, from 2% to 20% in twigs of *T. laxa* under high NaCl (Table 3). Such result suggested that in addition to the nutritional role, NO_3^- accumulation in the vacuoles could play an important role in balancing the osmotic potential in *T. laxa* under high salinity with adequate NO_3^--N supply.

The interaction between Cl^- and NO_3^- may strongly affect the contribution of both anions to osmotic regulation in plant. The use of more NO_3^- but less Cl^- ions for osmotic adjustment may prevent Cl^- toxicity in *Suaeda physophora* (Song et al. 2006a). Cl^- present in the expanded leaves of certain species is associated with chlorosis and death, and these injuries occur even when the Na^+ concentration is low in the leaves (Greenway and Munns 1983). However, in our present study, more NO_3^- but less Cl^- or Na^+ might be accumulated in the vacuole for osmotic adjustment at higher NO_3^- supply, compared with 1 mM NO_3^-. As NO_3^- supply increased, the decrease in the estimated contribution of Cl^- to osmotic potential in *T. laxa* was compensated by an increase in that of NO_3^- (Table 3). This can be attributed to competition between NO_3^- and Cl^- for transport systems, which are proposed to play significant roles in uptake or the xylem loading of NO_3^- and Cl^- (Cerezo et al. 1997; Köhler and Raschke 2000).

Many studies have shown that some N-containing organic compounds, like proline, glycinebetaine and other free amino acids, play crucial roles in osmotic balance in cytoplasm of plants under saline stress (Khedr et al. 2003; Parida and Das 2005). The accumulation of these organic osmolytes is believed to constitute "osmotic adjustment", as they promote water uptake from a hyperosmotic environment (Hasegawa et al. 2000; Maggio et al. 2002). In many species, the concentration of proline can also be used as a biomarker to indicate the extent of salinity stress (Bar-Nun and Poljaoff-Mayber 1977). It is assumed that accumulating organic solutes in the cytoplasm demands more energy than accumulating inorganic ions (Greenway and Munns 1983; Munns 2002). Accumulation of the salt ions in the vacuole to attain the cell osmotic balance under salt stress is a successful mechanism. Cells, in fact, strongly reduce their need to invest valuable metabolites in the synthesis of organic osmolytes (Di Martino et al. 2003). In the present study, the concentrations of proline increased significantly with increased NaCl level under adequate nitrate supply. However, the biosynthesis of proline decreased when NO_3^- supply increased to 10 mM NO_3^--N at 300 mM NaCl (Fig. 3). The contribution of proline to the Ψs under 300 mM NaCl declined as NO_3^--N supply increased from 1 to 10 mM, while the contribution of nitrate to the Ψs was enhanced (Table 3). Such results suggested that proline may not play an important role in osmoregulation, and higher accumulation of nitrate in the vacuole alleviated the effects of salinity stress on the plant by balancing the osmotic potential.

Saline soils are affected by the presence of soluble salts, with or without high amounts of exchangeable sodium. Different types of saline soils contain different cations (e.g. Na^+, K^+, Ca^{2+}, Mg^{2+}) and anions (Cl^-, SO_4^{2-}, CO_3^{2-}, HCO_3^-). Nitrate has been considered as a factor in the salinity of some soils (Caldwell 1974). However, some saline soils contain much higher nitrate levels (2–8 g / kg in 0–100 cm), and many plant species grow in these soils (Wang et al. 1993; Xi et al. 2006; Yang 1999). Our findings might partially explain the adaptation strategy taken by plants growing in such 'nitric saline soils'. In conclusion, an adequate NO_3^--N supply might have an important role to play in osmotic adjustment by the recretohalophyte *T. laxa* exposed into high salinity.

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