Acta Physiol Plant (2011) 33:963–971
DOI 10.1007/s11738-010-0628-1

ORIGINAL PAPER

Influence of NaCl-salinity on growth, photosynthesis, water relations and solute accumulation in *Phragmites australis*

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Received: 23 February 2010 / Revised: 21 September 2010 / Accepted: 12 October 2010 / Published online: 27 October 2010

Abstract The aim of this study was to investigate the effects of NaCl-salinity on the physiological attributes in common reed, *Phragmites australis* (Cav.) Trin. ex Steudel. Plants grew optimally under salinity treatment with standard nutrient solution without added salt and at NaCl concentrations up to 100 mM. Applied for 21 days, NaCl-salinity (300 and 500 mM) caused a significant reduction in growth allocation of all different tissues of *P. australis*. Shoot growth of reed plants displayed a highly significant correlation with plant–water relations and photosynthetic parameters. The net photosynthetic rate and stomatal conductance of reed plants treated with NaCl-salinity at varying osmotic potential (ψ_π) of nutrient solutions were positively correlated, and the former variable also had a strong positive relationship with transpiration rate. Leaf water potential and ψ_π followed similar trends and declined significantly as ψ_π of watering solutions was lowered. The increase in total inorganic nutrients resulting from increased Na^+ and Cl^- in all tissues and K^+, Ca^{2+} and Mg^{2+} concentrations were maintained even at the most extreme salt concentration. Common reed exhibited high K^+/Na^+ and Ca^{2+}/Na^+ selectivity ratios over a wide range of salinities under NaCl-salinity. These findings suggest that reed plants were able to adapt well to high salinities by lowering their leaf ψ_π and the adjustment of osmotically active solutes in the leaves.

Keywords Ion accumulation · Osmotic adjustment · Photosynthesis · *Phragmites australis* · Proline · Relative growth rate · Salinity · Water relations

Abbreviations

C_i	Internal CO_2 concentration
DM	Dry mass
E	Transpiration rate
FM	Fresh mass
g_s	Stomatal conductance
P_N	Net photosynthetic rate
RGR	Relative growth rate
$S_{K/Na}$	K^+/Na^+ selectivity ratio
$S_{Ca/Na}$	Ca^{2+}/Na^+ selectivity ratio
ψ_π	Osmotic potential
ψ_p	Turgor potential
ψ_w	Water potential
WUE	Water use efficiency
WUE_i	Intrinsic WUE

Introduction

The deleterious effects of salinity on plant growth are associated with low osmotic potential (ψ_π) of soil solution, nutritional imbalance, specific ion effect, hormonal imbalance and induction of oxidative stress, or a combination of these factors (Greenway and Munns 1980; Marschner 1995; Parida and Das 2005). Responses of species to salt stress depend on several interacting

Communicated by S. Renault.

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variables, including the magnitude (salt concentration and time of exposure) of the stress, plant genotype, plant developmental stage and cultural environment (Sultana et al. 1999; Jaleel et al. 2007). As indicated by Munns (1993), the effect of salinity on plant growth occurs in two-phase model. The first phase of the growth reduction is due to the osmotic stress of the salt outside the roots, the second phase being due to toxic effects, that is to the accumulation of salt inside the plant to toxic levels. However, by comparing two maize lines with different salt-tolerance, Zhao et al. (2010) showed that the growth response was simultaneously inhibited by ionic and osmotic effects for the two-phase model. Salt tolerance is not exclusively correlated with adaptation to Na^+ or Cl^- toxicity per se but also reflects adaptations to secondary effects of salinity (Munns 2002). Potassium uptake is particularly important due to the chemical similarities between Na^+ and K^+, which makes difficult the discrimination between the two ions by transport proteins (Blumwald et al. 2000). Thus, high levels of Na^+, or high Na^+:K^+ ratios can disrupt various enzymatic processes in the cytoplasm. Therefore, one of the key elements in salinity tolerance is the ability to maintain a high cytosolic K^+/Na^+ selectivity ($S_{K/Na}$) (Yeo 1998; Maathuis and Amtmann 1999; Blumwald et al. 2000).

Plant biomass production depends on the accumulation of carbon products through photosynthesis, but elevated salinity can adversely affect photosynthesis (Zhu 2001; Ashraf 2003). A decline in photosynthetic capacity of many plant species in response to salt stress has been ascribed to either stomatal, restricting CO_2 entry into leaves (Steduto et al. 2000; Meloni et al. 2003), or non-stomatal limitation that results in inhibition or down-regulation of photosynthesis (Dunn and Neales 1993; Sultana et al. 1999). The presence of salt decreases the ψ_π of the medium, so plants have problems with respect to absorption of water. In order to compensate for the negative values of the nutrient solution, plants have to decrease their water potential (ψ_w); this involves a decrease of the ψ_π, to maintain turgor and achieve osmotic adjustment (Blum et al. 1996). Salinity reduces the ability of plants to take up water, causing a reduction in growth along with a suite of metabolic changes (Munns 2002). A metabolic response to salt stress is the synthesis of compatible osmolytes (Ashraf and Foolad 2007). These mediate osmotic adjustment and therefore protect sub-cellular structures and reduce oxidative damage caused by free radicals, produced in response to high salinity (Zhu 2001).

Phragmites australis (Cav.) Trin. ex Steudel (synonymous to *P. communis* Trin.), common reed, is a widespread species in the temperate regions of the world (Den Hartog et al. 1989). Its typical habitats are fresh and brackish water areas of swamps, riversides, and lakesides. It is often the key-species in wetland ecosystems and propagates both through rhizome growth and seed germination in sparsely populated patches (Gorai et al. 2006). However, reed plants have adapted to terrestrial habitats, and various ecotypes have evolved with resistance to drought, salinity, and low temperature (Matoh et al. 1988; Wang et al. 1998; Pagter et al. 2005; Gorai et al. 2007; Engloner 2009; Gorai 2009). Among these, salinity is a well-known stressor of *P. australis*, leading to reduced vigour and success in brackish and salt marshes (Burdick et al. 2001). The aim of the present study was to investigate, for *P. australis* plants grown under greenhouse conditions, whether NaCl-salinity at varying ψ_π of nutrient solutions was related to growth, leaf gas exchange, water (and ion) relations and osmotic adjustment. Additionally, the correlation between growth and different physiological attributes was evaluated.

Materials and methods

Plant material and culture conditions

Seeds of *Phragmites australis* were collected in November 2003 from a saline location in Zerkine, Gabès (33°43′N, 10°16′E; southeast Tunisia). One thousand seeds weighed, on average, 100 mg (Gorai et al. 2007). This area is arid to semi-arid with a typical Mediterranean climate, characterized by irregular rainfall events and a harsh dry summer period. Annual rainfall is around 187 mm and annual mean evapo-transpiration 996 mm. Mean annual temperature is 19.3°C with a minimum temperature 5.9°C in January and 32.7°C maximum in August.

Seeds were surface sterilized with sodium hypochlorite solution for 1 min and germinated on filter paper in 90 mm Petri dishes at controlled conditions (Gorai et al. 2006). Seedlings were transferred to 3 l-plastic tanks for hydroponic growth, using aerated Hewitt nutrient solution (Hewitt 1966), containing macronutrients (mM): $MgSO_4$ (1.5), KH_2PO_4 (1.6), K_2HPO_4 (0.4), KNO_3 (3), NH_4NO_3 (2), $Ca(NO_3)_2$ (3.5). The medium contained also iron as complex EDTA–K–Fe (Jacobson 1951) and micronutrients as a mixture of salts: $MnCl_2$; $CuSO_4$, $5H_2O$; $ZnSO_4$, $7H_2O$; $Mo_7O_{24}(NH_4)_6$, $4H_2O$ and H_3BO_3 (Arnon and Hoagland 1940). Plants were grown in a growth chamber under the following conditions: 25 ± 1°C temperature, 50% day and 75% night relative humidity and 16 h light/8 h dark regime with 250 µmol m^{-2} s^{-1} photosynthetic active radiations (PAR). The nutrient solutions were replaced after every 3rd day. The solution pH was adjusted to 6.5 ± 0.1 every day with NaOH or HCl, as required.

The experiment was arranged in a growth chamber in a completely randomized design with four NaCl-salinity treatments × six replicates. Plants were supplied with a control nutrient solution (0 mM NaCl, $\psi_\pi = -0.04$ MPa,)

or saline nutrient solutions (100, 300 and 500 mM NaCl) at varying ψ_π of −0.53, −1.51 and −2.49 MPa, respectively. The osmolality of the solutions was analysed using a vapour pressure osmometer (Wescor 5520, Logan, UT, USA). To avoid osmotic shock, the final salt concentration (100, 300 or 500 mM) was progressively adjusted by adding NaCl of 50 mM twice daily. Two harvests were made, at the beginning of treatment (2-month-old plants) and 21 days later. At the harvests, leaves, stems and roots separated from rhizomes were successively rinsed three times in cold water and blotted between two layers of filter-paper. The fresh mass (FM) was measured immediately, and the dry mass (DM) after 48 h of desiccation in an oven at 60°C. Plant relative growth rate (RGR) was determined as $RGR = \Delta M/\underline{M}\ \Delta t$, where Δ is the difference between values at the final and initial harvests, t is the time (days) and M is the whole plant DM (g). $\underline{M}$ is the logarithmic mean of M calculated over the Δt period (Hunt 1990): $\underline{M} = \Delta M/\Delta \ln(M)$.

Leaf water relations

The water content (WC) of different tissues was determined as WC (g H_2O g^{-1} DM) = (FM − DM)/DM. Leaf ψ_w was measured using a pressure chamber (PMS Instruments Co., Corvallis, OR, USA) after 21 days of salt treatment, according to Scholander et al. (1965). After measuring of ψ_w, the samples were frozen in liquid nitrogen and stored at −20°C. Leaf tissues were thawed and centrifuged at 1,200×g for 25 min at 4°C to extract the cell sap. A vapour pressure osmometer (Wescor 5520, Logan, UT, USA) was used to determine osmolality of the sap expressed from leaves, which was converted to ψ_π, by the van't Hoff equation: $\psi_\pi = -ciRT$, where ci is the value reading from the instrument, R is the ideal gas constant and T is the absolute temperature (Nobel 1991). Turgor potential (ψ_p) was determined using the relationship: $\psi_p = \psi_w - \psi_\pi$.

CO_2 and H_2O gas-exchange

After 21 days at 0, 100, 300 and 500 mM NaCl, photosynthetic gas exchange parameters were measured between 10:00 and 12:00 h using an LCpro + portable photosynthesis system (ADC, BioScientific Ltd, UK). The CO_2 concentration in the leaf chamber was set at 360 µmol mol^{-1}. The leaf was irradiated with PAR of 1,500 µmol m^{-2} s^{-1} of internal light source. The third youngest fully expanded leaf was used for these measurements. Readings were logged every 30 s until stable values for net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and internal CO_2 concentration (C_i) were reached. The water use efficiency (WUE) and intrinsic WUE (WUE_i) were calculated as P_N/E and P_N/g_s, respectively.

Organic and inorganic solute determination

The proline was quantified spectrophotometrically by the ninhydrin method according to Bates et al. (1973). The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 14,000 rpm. The supernatant was used for the estimation of the proline concentration. The reaction mixture consisted of 2 ml of acid ninhydrin and 2 ml of glacial acetic acid, which was boiled at 100°C for 1 h. After termination of reaction in ice bath, the reaction mixture was extracted with 4 ml of toluene, and absorbance was read at 520 nm. The leaf proline concentration was expressed on dry weight basis.

Ions were extracted from dried, milled plant material with nitric acid (HNO_3, 0.5%). Concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} were determined using an atomic absorption spectrophotometer (Schimazu AA 6800, Schimazu Crop, Kyoto, Japan), while Cl^- concentration was determined on the same extract with a chloride meter (Jenway PC LM3, London, UK).

K^+/Na^+ and Ca^{2+}/Na^+ selectivity ratios

The selectivity ratios of K^+ and Ca^{2+} over Na^+ ($S_{K/Na}$, $S_{Ca/Na}$) for accumulation, uptake and transport were estimated as: $[K \text{ or } Ca/Na]_{whole\ plant}/[K \text{ or } Ca/Na]_{medium}$, $[K \text{ or } Ca/Na]_{root}/[K \text{ or } Ca/Na]_{medium}$ and $[K \text{ or } Ca/Na]_{shoot}/[K \text{ or } Ca/Na]_{root}$, respectively (Gorai et al. 2010). K, Ca and Na represent, respectively, the quantity found in the whole plant (accumulation), roots (uptake) and shoots (transport). In the medium and roots, rather it is concentration.

Statistical analysis

Data were analysed using SPSS statistical package (SPSS 2002). Data were tested for normal distribution using the Shapiro–Wilk Test, and heterogeneities of variance within treatments were tested using Levene's Test. When necessary, log transformations were used to normalize distributions. Student–Newman–Keuls test was used to estimate least significant range between means. Correlation coefficients between data were examined using Pearson's correlation coefficient at or below the 5% significance level.

Results

Growth analysis

A one-way ANOVA on the biomass production of *P. australis* indicated that salinity significantly affected DM ($P < 0.001$). Root dry mass increased significantly in salinity of 100 mM NaCl, but yield of roots progressively

declined with further increases in salinity (Fig. 1a). The RGR followed a similar trend that dry matter allocation and decreased significantly in all tissues ($P < 0.001$) at the highest NaCl concentration. At all levels of salinity, leaves had the lowest growth activity. Root growth was less sensitive to NaCl than was shoot growth, translating a decrease in dry matter allocation to the shoots (Fig. 1b). At 500 mM NaCl, the fresh:dry weight ratio for all tissues was significantly decreased compared with lower salinity and we recorded 60, 59 and 65% of controls, respectively, for leaves, stems and roots (Fig. 1c).

Water relations

Salinity significantly affected tissue WC of *P. australis* tissues ($P < 0.001$). In all treatments, WC was lower in stems and leaves than in roots. Root WC increased slightly at 100 mM NaCl, but declined at higher salinities (Fig. 1d). All leaf water relation parameters decreased with increasing NaCl concentration (Fig. 2a–c). A one-way ANOVA of the water status of *P. australis* revealed that salinity significantly affected ψ_w, ψ_π and ψ_p ($P < 0.001$) of salinized plant leaves relative to control ones. Linear regression analysis was used to determine the relationships between leaf water relations and ψ_π of the watering solutions. There was a strong positive relationship between ψ_π of solutions and ψ_w or ψ_π of leaves (Fig. 2a, b), with R^2 values 0.96 and 0.95, respectively, while a negative correlation was identified between ψ_π of solutions and leaf ψ_p, with $R^2 = 0.66$ (Fig. 2c). This decrease in water relations of *P. australis* plants under salt stress indicated dehydration and turgor loss.

CO_2 exchange, transpiration and stomatal conductance

The P_N, g_s, E and C_i were significantly affected by NaCl treatment (Fig. 3a–d). There was a significant negative relationship between water potential of solutions and P_N, g_s, E and C_i, with coefficient of correlation (R^2) values of 0.75, 0.79, 0.78 and 0.46, respectively. The P_N significantly decreased with increase in NaCl concentration ($P < 0.001$). It was reduced from 10.9 μmol m^{-2} s^{-1} in control plants to an approximately fivefold lower rate in the most stressed plants (Fig. 3d). The C_i was slightly reduced in salinized plants, as compared with controls. This concentration did not differ statistically between control plants and those at 100 mM NaCl (Fig. 3a). The E was severely decreased with increase in NaCl concentration in the nutrient solution. The highest rate was recorded in control plants (3.46 mmol m^{-2} s^{-1}), and approximately sevenfold lower rate in plants stressed at 500 mM NaCl (Fig. 3b). The decrease in g_s was more than those in P_N and E with increasing NaCl concentration (Fig. 3c). As illustrated in Fig. 3e and f, salinity treatment with decreasing ψ_π of solutions had a significant effect on WUE_i ($P < 0.05$, $R^2 = 0.36$) as compared with controls; however, the WUE values did not differ significantly ($P > 0.05$, $R^2 = 0.10$).

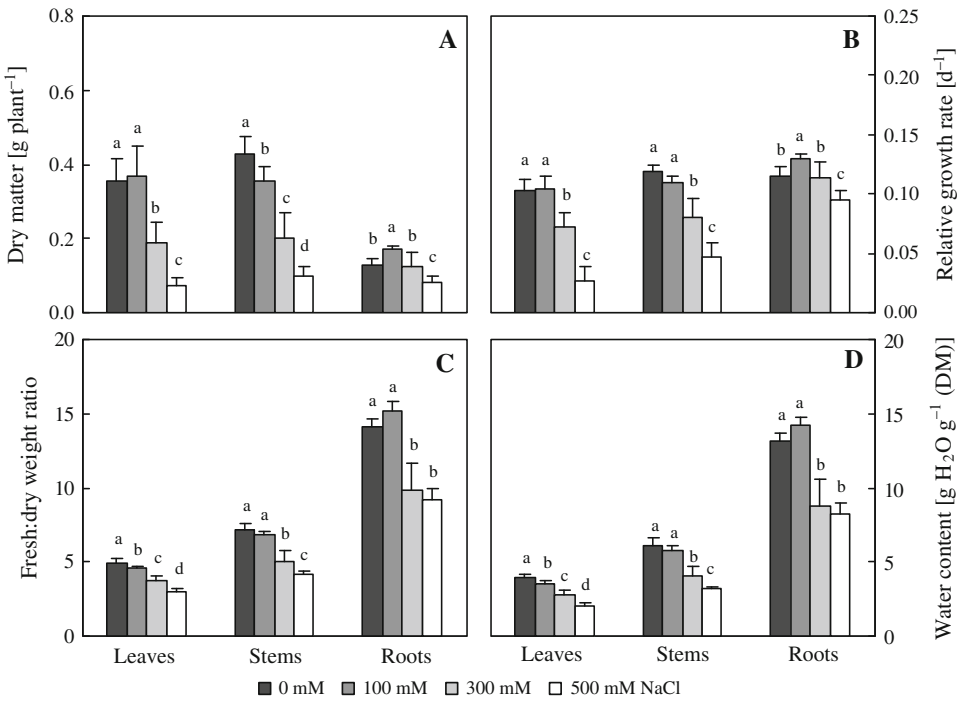

Fig. 1 Changes in tissue **a** dry mass, **b** relative growth rate, **c** fresh:dry weight ratio and **d** water content of *Phragmites australis* when 2-month old plants were subjected for 21 days to salinity at varying NaCl concentrations (0, 100, 300 or 500 mM) in nutrient solution. Data represent means ± SE ($n = 6$). Different *letters* indicate significant differences between treatments at $P < 0.05$ according to the Student–Newman–Keuls test

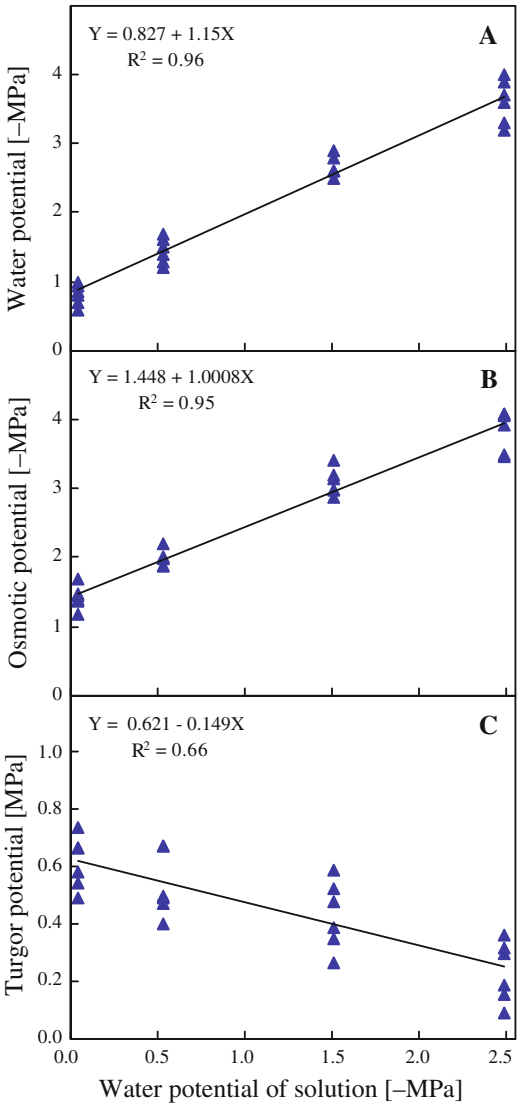

Fig. 2 Regression plots for **a** water potential, ψ_w; **b** osmotic potential, ψ_π; and **c** turgor potential, ψ_p in leaves of *Phragmites australis* when 2-month-old plants were subjected for 21 days to salinity with a standard nutrient solution ($\psi_\pi = -0.04$ MPa) or NaCl solutions of various osmotic potentials (−0.53, −1.51 or −2.49 MPa). *Lines* describing the evolution of each parameter were obtained using a linear regression. Values are from the four treatments with six replicates ($n = 24$)

Correlations between growth and physiological attributes

Table 1 shows the correlation coefficients between growth and different physiological attributes. As expected, high correlation coefficients were found between growth (shoot DM and shoot RGR) and all photosynthetic parameters of *P. australis* plants except WUE, when exposed to salinity at varying ψ_π of watering solutions (Table 1). Additionally, the correlation coefficients between shoot DM and leaf water relations were significantly high, suggesting strong relationships (shoot DM × ψ_w, $R^2 = 0.92$; shoot DM × ψ_π, $R^2 = 0.92$; shoot DM × ψ_p, $R^2 = 0.72$).

Inorganic and organic solute concentrations

A two-way ANOVA showed significant individual effects of plant tissue, salinity treatment and their interactions on ion concentrations (Table 2). Reed plants cultivated under varying NaCl concentrations showed significantly higher Na^+ accumulation in the stems ($P < 0.001$), as compared with that in the leaves ($P < 0.001$) and the roots ($P < 0.001$) (Fig. 4a). Stem Na^+ concentration ranged from 0.018 to 0.814 mol l^{-1} at 100 and 500 mM NaCl, respectively, whereas those of leaves and roots varied from 0.017 to 0.518 mol l^{-1} and 0.011 to 0.378 mol l^{-1}, respectively (Fig. 4a). Chloride concentration showed similar changes, but at lower levels than sodium (P values < 0.001; Fig. 4b). Potassium concentration in leaves significantly increased at 500 mM NaCl, but there were no differences in both stems and roots with increasing NaCl supply (Fig. 4c). As to Ca^{2+} and Mg^{2+} accumulation, NaCl treatment had no effect on concentrations of these ions in leaves at salinity levels of 0–300 mM NaCl, and differences were significantly increased at the highest concentration. Increasing NaCl-salinity had no adverse effect on Ca^{2+} and Mg^{2+} concentrations in stems and roots and high amounts were reached at a NaCl concentration of 500 mM (Fig. 4d–e).

As shown in Fig. 4f, proline concentration in leaves of *P. australis* was significantly increased by solutions of increased osmolality ($P < 0.001$). The leaves of plants subjected to 500 mM NaCl accumulated sevenfold more proline than the controls.

Figure 5 shows the selective accumulation of K^+ and Ca^{2+} over Na^+ estimated by the K^+/Na^+ and Ca^{2+}/Na^+ ratios of these ions in the culture medium and *P. australis* organs. The $S_{K/Na}$ and $S_{Ca/Na}$ for accumulation ranged from 27.78 to 34.70 and from 6.01 to 9.52 at 100 and 500 mM NaCl, respectively, whereas that for uptake varied from 16.2 to 20.6 and from 2.36 to 5.62, respectively. However, the $S_{K/Na}$ and $S_{Ca/Na}$ for transport in shoots were lower than those of uptake and accumulation (Fig. 5).

Discussion

Common reed's growth decreased progressively with increase in NaCl concentration in the nutrient solution. This was in agreement with previous reports (Gorai et al. 2007, 2010; Pagter et al. 2009). The depressive effect of salinity on plant growth is commonly due to both osmotic and ion-specific effects (Greenway and Munns 1980; Munns 2002). It has been reported that reductions in

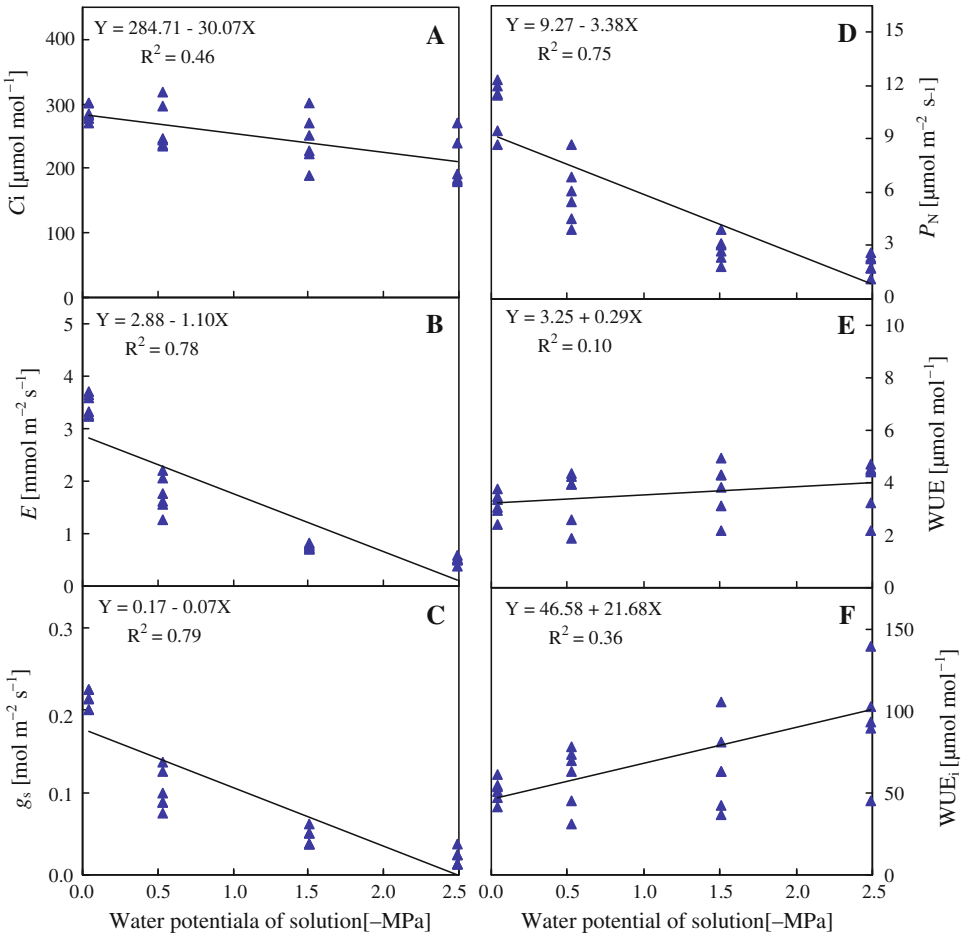

Fig. 3 Changes in **a** internal CO_2 concentration, C_i; **b** transpiration rate, E; **c** stomatal conductance, g_s; **d** net photosynthetic rate, P_N; **e** water use efficiency, WUE; and **f** intrinsic WUE, WUE_i of *Phragmites australis* when 2-month-old plants were subjected for 21 days to salinity with a standard nutrient solution ($\psi_\pi = -0.04$ MPa) or NaCl solutions of various osmotic potentials (−0.53, −1.51 or −2.49 MPa). Lines describing the dependencies were obtained using a linear regression. Values are from four treatments with six replicates ($n = 24$)

growth depend on the period of time over which the plants have grown in saline conditions, leading to the Munns' two-phase hypothesis in response to salt stress. During the period of treatment, reed plants showed a two-phase growth response to salinity.

Several research reports that plants grown under salt stress manifest acclimation to success their establishment, by lowering both leaf ψ_w and ψ_π (Sultana et al. 1999; Koyro 2006). Osmotic adjustment by net accumulation of solutes in cells in response to a fall in the ψ_w of their environment can in part offset this deterioration of growth conditions. As a consequence of this net accumulation, the cell ψ_π is lowered, and turgor pressure tends to be maintained (Blum et al. 1996). In the present experiment, increasing salinity was accompanied by a decrease in WC, leaf ψ_w and ψ_π of reed plants. Thus, turgor could be maintained, and obviously the osmotic adjustment was sufficient to compensate the reduction in leaf ψ_w in these reed plants growing under salinity conditions. According to Gorai et al. (2010), leaf ψ_w and ψ_π of reed plants exposed to hypoxia at varying NaCl-salinity concentrations declined significantly as ψ_π of watering solutions was lowered.

Photosynthetic activity is one of the major factors controlling growth (Tezara et al. 2002; Ashraf 2004). The P_N presented here clearly shows a correlation with shoot growth of *P. australis* treated with increased osmolarity of solutions. The present study depicts that P_N and g_s of *P. australis* under salt stress were positively associated and the former variable also had a strong positive relationship with E. A similar result was obtained by Pagter et al. (2009) showing that stomatal conductance in *P. australis* was negatively influenced by decreasing ψ_π of nutrient solutions caused by NaCl or Na_2SO_4. Choi et al. (2005) showed that carbon isotope discrimination in *P. australis* grown in a constructed saline wetland decreased as salinity increased, indicating a decline in stomatal and/or mesophyll conductance. Working on the same species, Wang et al. (1998), Lissner et al. (1999), Pagter et al. (2009) and Gorai et al. (2010) showed that increasing osmolarity of growth medium affects P_N not only due to its effects on stomatal regulation but also due to other non-stomatal responses. In reed plants subjected to hypoxia at various ψ_π, Gorai et al. (2010) showed no clear relationships between growth and photosynthetic parameters except for

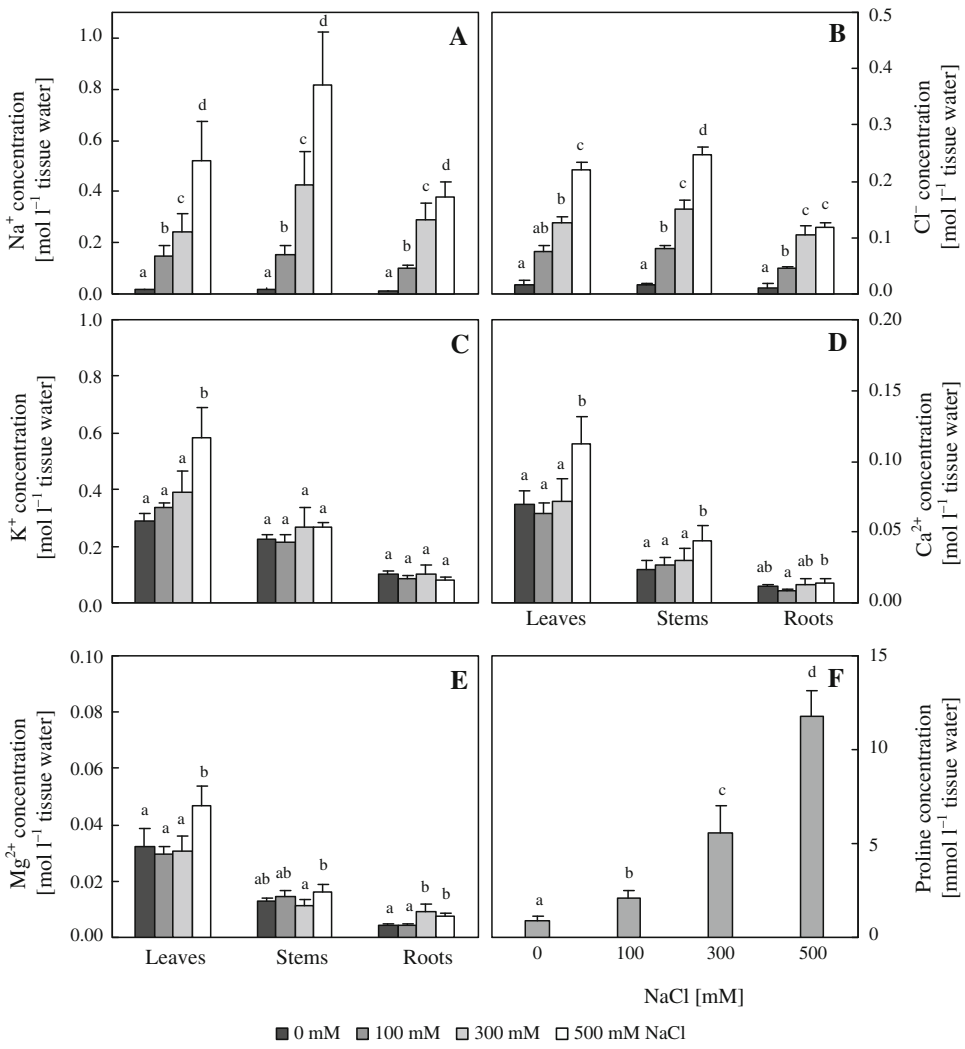

Fig. 4 Changes in Na^+ (**a**), Cl^- (**b**), K^+ (**c**), Ca^{2+} (**d**) and Mg^{2+} (**e**) concentrations in leaves, stems and roots and proline (**f**) concentration in leaves of *Phragmites australis* grown on nutrient solution supplied with different NaCl concentrations (0, 100, 300 or 500 mM). Concentration was calculated on a tissue water basis. Data represent means ± SE ($n = 6$). Different *letters* indicate significant differences between treatments at $P < 0.05$ according to the Student–Newman–Keuls test

Table 1 Correlation coefficients (R^2) between pairs of growth and physiological attributes of *Phragmites australis* when 2-month-old plants were subjected for 21 days to salt stress at varying NaCl concentrations (0, 100, 300 or 500 mM) in nutrient solution

Parameters	Shoot DM	Shoot RGR	P_N	C_i	E	g_s	WUE_i	WUE	ψ_w	ψ_π
Shoot RGR	0.97									
P_N	0.81	0.76								
C_i	0.54	0.59	0.49							
E	0.77	0.74	0.94	0.67						
g_s	0.80	0.77	0.95	0.64	0.98					
WUE_i	−0.52	−0.57	*−0.37*	−0.45	−0.44	−0.55				
WUE	*−0.20*	*−0.20*	*−0.11*	−0.58	*−0.39*	*−0.35*	0.48			
ψ_w	0.92	0.93	0.87	0.69	0.88	0.90	−0.59	*−0.28*		
ψ_π	0.92	0.93	0.84	0.67	0.89	0.90	-0.56	*−0.28*	0.99	
ψ_p	0.72	0.72	0.66	0.66	0.67	0.70	−0.65	*−0.25*	0.83	0.76

Italic values are not significant at $P < 0.05$; df ($n = 30$)

g_s, whereas growth displayed a highly significant correlation with plant-water relations.

Sodium contribution to the total amount of cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) became important with an increase in NaCl-salinity level and more marked in the shoots, as compared with the roots. There are several reports on considerable inhibition of nutrient uptake, notably K^+, Ca^{2+} and Mg^{2+} uptake under salinity (Marschner 1995;

Table 2 Results of two-way analysis of variance of plant characteristics by salinity (S), plant tissues (PT) and their interaction (S × T)

Dependent variable	Main factors		Interaction (S × T)
	Salinity (*S*)	Plant tissue (PT)	
Na^+	79.69**	12.38**	4.84**
Cl^-	55.61**	9.35**	2.48^{ns}
K^+	12.32**	173.55**	9.44**
Ca^{2+}	16.18**	215.58**	5.16**
Mg^{2+}	10.53**	254.48**	5.73**

ns non-significant

Numbers are *F*-values significant at * $P < 0.05$, ** $P < 0.001$

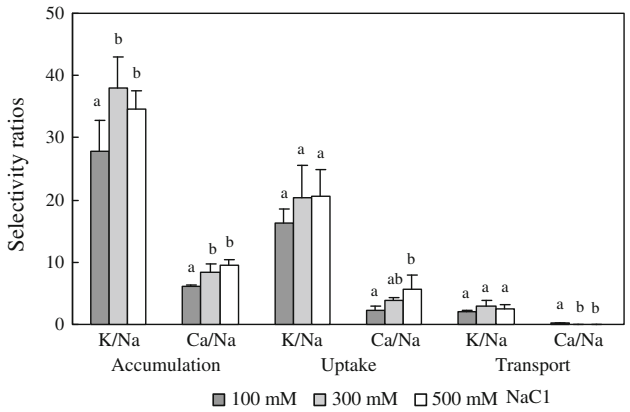

Fig. 5 Changes in the K^+/Na^+ and Ca^{2+}/Na^+ selectivity ratios for accumulation, uptake and transport in *Phragmites australis* when 2-month-old plants were subjected for 21 days to salinity at varying NaCl concentrations (0, 100, 300 or 500 mM) in nutrient solution. Data represent means ± SE ($n = 6$). Different *letters* indicate significant differences between treatments at $P < 0.05$ according to the Student–Newman–Keuls test

Munns 2002; Netondo et al. 2004). The common reed, in contrast, showed increased accumulation of shoot K^+ and root and shoot Ca^{2+} and Mg^{2+} concentrations, and therefore able to adapt well to high salinities. The present data agree with findings reported on *Cynodon dactylon* (Hameed and Ashraf 2008) in which concentrations of K^+ and Ca^{2+} in the roots as well as shoots increased with an increase of salt level. *Phragmites australis* appears, thus, able to maintain a high $S_{K/Na}$ for accumulation in the whole plant and the uptake by roots; however, the transport in shoots reached lower values when salinity increases in the medium. Many studies on halophytes and some tolerant glycophytes plants showed that a high $S_{K/Na}$ for transport is a salt tolerance criterion (Gorham et al. 1990; Shachtman et al. 1991; Wolf et al. 1991; Yeo 1998). Selectivity ratio for transport of K^+ over Na^+ in *Cakile maritima*, a halophytic species occurring on dunes along the Tunisian seashore (Debez et al. 2004) was 3.5-fold higher than in common reed (present study) at a NaCl concentration of 500 mM. Yeo (1998) suggested that dry matter production is proportional to leaf K^+ nutrition. The capacity of plants to counteract salinity stress strongly depends on the status of their potassium nutrition (Zhu 2001). The ability of reed species to maintain a substantial growth rate under saline conditions is directly related to an efficient $S_{K/Na}$ and $S_{Ca/Na}$. Moreover, *P. australis* tolerance was due to its capacity to limit Na^+ transport and to enhance K^+ supply to the shoots. Between different ecotypes of reed plants differences in NaCl tolerance have been ascribed to differences in ion selectivity of a high-affinity plasma membrane K^+ transporter resulting in higher K/Na ratios in salt-tolerant than in salt-sensitive ecotypes (Takahashi et al. 2007). Common reed has an efficient mechanism of Na^+ exclusion from the leaves and exhibited a high leaf $S_{K/Na}$ over a wide range of salinities under hypoxia treatment (Gorai et al. 2010).

Proline is a compatible solute that accumulates in response to osmotic stress, and the accumulation of this osmolyte represents an important adaptive response to salt and drought stress (Ashraf and Harris 2004; Parida and Das 2005). Reed plants subjected to salt stress accumulated sevenfold more proline than controls. There was a strong positive correlation between proline concentration and osmolality in the leaves of *P. australis* in response to NaCl concentrations in the culture medium.

Overall, common reed's growth declined significantly as water potential of watering solutions was lowered. Shoot growth was positively associated with a decrease in leaf gas exchange characteristics and water potential components. The g_s was reduced linearly as ψ_π of solutions was decreased and influenced accordingly P_N and E. Plants were able to adapt well to salinity at varying ψ_π of nutrient solutions by lowering their leaf ψ_π. Reduction in the leaf ψ_w was achieved by the adjustment of osmotically active solutes in the leaves.

Acknowledgments We are thankful to the journal associate editor Prof. Sylvie Renault and three anonymous referees for their constructive comments on earlier versions of the manuscript.

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