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## Moderate salinity stimulates growth and photosynthesis of *Phragmites karka* by water relations and tissue specific ion regulation



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### ABSTRACT

The domestication of non-food salt resistant plants could be helpful for biofuel as well as phytoremediation of salt-affected lands. Limited eco-physiological studies on halophytes are the major bottleneck in developing such plants as energy crops. The aim of the present work was to determine the effect of salt stress on growth, ion homeostasis, water status and photosynthesis of *Phragmites karka* – a potential biofuel crop. Plants were grown in 0, 100 and 300 mM NaCl (equivalent to non-saline, 20 and 60% seawater). Our results indicate that the addition of 100 mM NaCl to the growth medium increased plant biomass. Leaf osmotic adjustment was primarily achieved by inorganic solutes (K<sup>+</sup> and Na<sup>+</sup>) while soluble sugars and proline remained unchanged. Higher K<sup>+</sup> selectivity and Na<sup>+</sup> exclusion from shoots improved photosynthesis and growth under moderately saline conditions. Decreased growth in 300 mM NaCl could be attributed to leaf tissue dehydration and turgor loss leading to stomatal closure and decreased CO<sub>2</sub> absorption. Our results indicate that *P. karka* could produce high ligno-cellulosic biomass for ethanol production using brackish water irrigation on saline soils avoiding competition with conventional agriculture.

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### 1. Introduction

Plant salt resistance involves physiological and biochemical changes which help them survive under stressed conditions (Marschner et al., 1996; Munns, 2002; Koyro, 2003). To observe these changes, four aspects are extensively studied: (a) growth rate and plant morphology, (b) resistance to water stress (reduction of the water potential), (c) regulation of CO<sub>2</sub> and H<sub>2</sub>O-exchange by stomata and (d) avoidance of ion toxicity and nutrient imbalance. Plants achieve an osmotic balance by accumulating inorganic ions (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>) and organic solutes, such as

**Abbreviations:** C, carbon; FW, fresh weights; KUE, potassium use efficiency; N, nitrogen; NUE, nitrogen use efficiency; WUE, water use efficiency.

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proline (Raymond and Smirnoff, 2002), glycine betaine (Rhodes and Hanson, 1993), polyamines (Gill and Tuteja, 2010), soluble sugars and polyols (Tattini et al., 1996). Two types of ionic responses to salt solution are known among grasses: Na<sup>+</sup> exclusion (Peng et al., 2004) and Na<sup>+</sup> accumulation (Bell and O Leary, 2003). The discrimination in utilization of K<sup>+</sup> and Na<sup>+</sup> under similar hydrated ionic radius could provide the basis for plant survival on salt affected soils (Benlloch et al., 1994). However, Na<sup>+</sup> which is considered toxic for most of the glycophytes could be effectively used for osmotic adjustment in halophytes (Marschner et al., 1996; Bell and O Leary, 2003). One reason could be the maintenance of osmotic potential as accumulation of Na<sup>+</sup> is inexpensive when compared to the metabolic costs for the synthesis of organic compounds (Koyro, 2006).

The regulation of Na<sup>+</sup> flux is highly controlled in salt resistant plants while absence of such capacity in salt-sensitive plants upsets ion homeostasis and can promote changes in plant metabolism (Takahashi et al., 2007). Regulation of Na<sup>+</sup> over K<sup>+</sup> selectivity is crucial for maintaining an adequate K<sup>+</sup>/Na<sup>+</sup> ratio in the cytoplasm leading to preserve biochemical and biophysical K<sup>+</sup> dependent

processes (Carden et al., 2003; Munns and Tester, 2008). Regulation of carbon flow from photosynthesis to osmolyte production, and utilization of energy to keep ion homeostasis as well as to promote biomass is crucial for the productive growth and survival of plant on saline habitats.

Sodium induced ion imbalance in chloroplasts results in reduced  $K^+$  availability and photosynthesis (Sudhir and Murthy, 2004). Excessive salt accumulation in roots and water stress, mediate ABA synthesis that leads to the stomatal closure (Montero et al., 1998). Non-stomatal limitations have been ascribed to sensitivity of photosystem II, reduced efficiency of Rubisco and reduced RuBP regeneration capacity (Muranaka et al., 2002).  $Na^+$  toxicity, reduced chlorophyll (Lutts et al., 1996) and stomatal conductance could impair net  $CO_2$  assimilation rate resulting in biomass reduction (Sudhir and Murthy, 2004; Koyro, 2006).

Most of the biofuel crops are salt sensitive (glycophytes) and are often used as food or fodder. An increasing demand for biofuel is currently competing with ideas on sustainable food production and protection of biodiversity (Foley et al., 2011). Increasing human population and a shift to higher meat and dairy utilization requires expansion in agricultural and forestry production (Foley et al., 2011). The declining fresh water supplies, increasing desertification and soil salinization have developed new interest in plant species possessing natural salt resistance, especially halophytes which are capable of producing economically profitable biomass (Khan et al., 2009; Koyro et al., 2013). One aspect of halophyte utilization is the production of energy (bioethanol, and biogas) from their biomass (Abideen et al., 2012; Gul et al., 2013). These biomass producing salt resistant plants need to be domesticated, and extensive studies would ensure sustainable growth conditions and optimal biomass production. Eco-physiological trials can uncover the mechanism of salt resistance, improvement in productivity for wide industrial application, and to assess their sustainability.

*Phragmites karka*, a perennial grass, is one of the dominant species distributed as pure population on saline marshy habitats around Karachi (Zehra and Khan, 2007). This species has the potential to become a biofuel crop because of its suitable lignocellulosic biomass (Abideen et al., 2012). In addition, this plant has the ability to withstand flooding that makes it an excellent stabilizer of erosion-prone river banks (Zehra and Khan, 2007). Seeds can germinate in up to 500 mM NaCl ( $\approx$ seawater), and plants can subsequently grow under saline conditions (Zehra and Khan, 2007). The present work was carried out to determine the effect of NaCl on growth, ion homeostasis, water status and photosynthesis of *P. karka* to determine its value as biofuel candidate under saline conditions.

## 2. Materials and methods

### 2.1. Growth conditions

Seeds of *P. karka* were collected in November 2011 from Karachi University campus, Pakistan. Experiments were conducted in an environmentally controlled greenhouse in Giessen, Germany (temperature:  $25 \pm 2^\circ C$ , relative humidity: 50% and photoperiod: 16/8 h day–night). The irradiation intensity was adjusted between 200 and  $250 \mu mol m^{-2} s^{-1}$  for plants. Seeds were sown in plastic pots (10 cm  $\times$  25 cm) filled with wet clay. Nutrients were provided by adding Wuxal Super (Aglukon, Düsseldorf, Germany) until the seedlings reached an age of 35 days. Seedlings were subsequently transplanted into plastic tubes (35 cm length, 11 cm diameter) containing 50% sand 30% clay and 20% gravel and exposed to periodic flooding (each day at 8 AM and 8 PM) in a quick check system (Koyro, 2003). Plants were irrigated with a permanently aerated basic nutrient solution (modified after Epstein, 1972). The

nutrient solutions were renewed after 30 days of acclimation before the onset of salinity treatments. Salinity of the nutrient solution was gradually increased (25 mM NaCl twice a day) until final concentrations of 100 and 300 mM NaCl were reached. These conditions were maintained for another five weeks (35 days). Data acquisition on leaf water relations and gas exchange were completed before final harvest for growth and other parameters.

### 2.2. Growth parameters

Plant height and number of green leaves were noted prior to the shoot and root separation. Fresh weights (FW) of root and shoot were recorded. Plant material was dried in an oven at  $80^\circ C$  until constant weight. A portion of the fresh samples were also immediately frozen in liquid nitrogen and stored at  $-20^\circ C$ . Leaf area (on a whole plant basis) was determined with a LICOR-3000C Portable Area Meter and a LI-3050C Transparent Belt Conveyor Accessory. All experiments were performed on three biological replicates.

### 2.3. Analysis of $Na^+$ , $K^+$ , $Mg^{2+}$ , $Ca^{2+}$ , N and C

Dried shoot and root samples (20 mg) were extracted with 10 ml of 0.5%  $HNO_3$  in a water bath ( $80^\circ C$ ) for 12 h. Concentration of  $Na^+$ ,  $K^+$ ,  $Mg^{2+}$  and  $Ca^{2+}$  were determined directly from the extract or of diluted samples with an atomic absorption spectrometer (AAS PE2100, Perkin Elmer).  $K^+/Na^+$  ratio was used as indicator of ion selectivity for absorption and transport (Pitman, 1965).

Potassium use efficiency (KUE) of leaves was determined by the following formula (Liu et al., 2000):

$$KUE = \frac{\text{Leaf dry weight}}{\text{Leaf K content}}$$

Total carbon (C) and nitrogen (N) contents (% dry weight) were determined in dried and homogenized leaf samples. About 20–200 mg samples were analyzed using CNS element analyzer (Vario MAX CNS, Elementar, Hanau, Germany). Nitrogen use efficiency of leaf (NUE) was determined by using the formula of Debez et al. (2006):

$$NUE = \frac{\text{Total plant dry weight}}{\text{Leaf N content}}$$

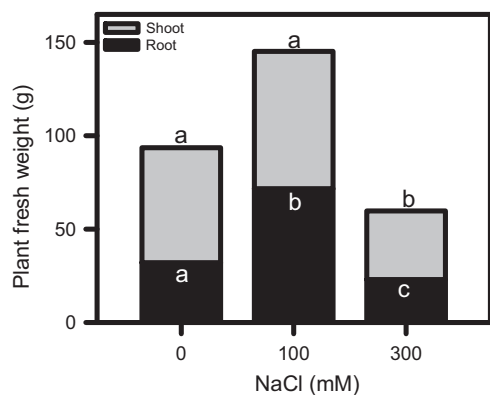
### 2.4. Leaf water relations

Predawn leaf water potential was measured via pressure chamber (Scholander et al., 1965) when the stomata were closed and water potential of plants was supposed to be equilibrated with soil water potential.

Osmotic values were determined from frozen leaf samples which were homogenized in a mortar and centrifuged (at  $4^\circ C$  for 5 min at  $3000 \times g$ ) to obtain press sap. The extracted sap was heated at  $50^\circ C$  for 2–3 min to denature enzymes. The osmotic values on treated press sap were determined by the freeze point depression cryo-osmometer (Osmomat 030, Gonotec). Osmotic potential was calculated using van't-Hoff equation described by Guerrier (1996):  $COP = -n * R * T$ ; where  $n$  is the number of moles of solute,  $R = 0.008314 J mol^{-1} K^{-1}$  (gas constant) and  $T = 298.8 K$  (absolute temperature).

### 2.5. Proline and sugar

Proline was extracted from 50 mg of leaf dry mass homogenized in 5 ml of 3% (v/v) sulfosalicylic acid. Samples were frozen in liquid  $N_2$  before grinding in a mortar and pestle. Proline was determined according to Bates et al. (1973) and expressed as mmol proline/kg dry weight.



**Fig. 1.** Tissue (shoot and root) per plant fresh weight of *Phragmites karka* treated with 0, 100 and 300 mM NaCl for 35 days. Mean values with different letters (black color for shoot and white color for root) indicate significant differences at  $P < 0.05$  (LSD).

Total soluble sugars in dried leaf powder were estimated using the method of Yemm and Willis (1954) and sucrose used as standard (Sigma–Aldrich).

## 2.6. Organic and inorganic osmotica

The percent contribution of leaf  $\text{Na}^+$ ,  $\text{K}^+$ , soluble sugars (as sucrose equivalents) and proline to leaf osmotic potential was calculated by Van't Hoff equation as described by Guerrier (1996).

## 2.7. Gas exchange and chlorophyll

Leaf gas exchange parameters were recorded using a portable infrared  $\text{CO}_2/\text{H}_2\text{O}$  gas exchange cuvette system LI-COR 6400 (LI-COR, Lincoln, NE, USA). Net photosynthetic rate, respiration, stomatal conductance, intercellular  $\text{CO}_2$  concentration, transpiration rate and water use efficiency ( $\text{WUE} = \text{net photosynthetic rate}/\text{stomatal conductance}$ ) were measured on fully expanded young leaf under saturating irradiation. The regression of the steady state light response curve was determined at photosynthetic photon flux density (PPFD) levels between 0 and  $1500 \mu\text{mol photon m}^{-2} \text{s}^{-1}$  using SigmaPlot 11 software. Estimated pigment content was analyzed using SPAD 502 (Konica Minolta, Japan).

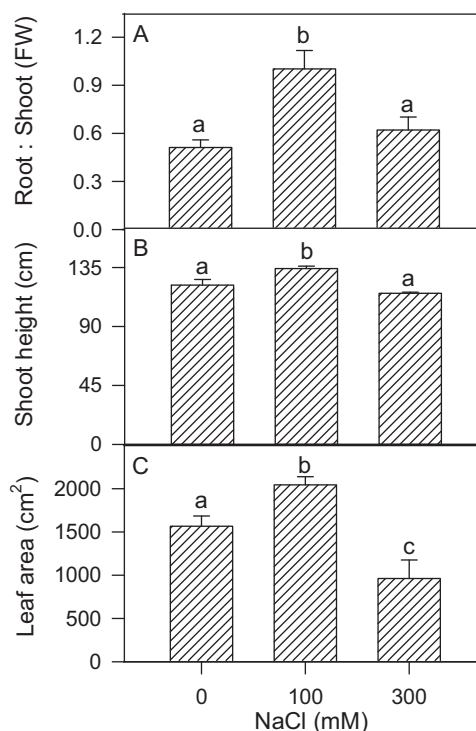
## 2.8. Statistical analysis

Data ( $n \geq 3$ ) were examined by one-way analysis of variance using SPSS (ver. 11) software and significant differences among means ( $P < 0.05$ ) were assessed by Fisher's protected least significance difference (LSD).

## 3. Results

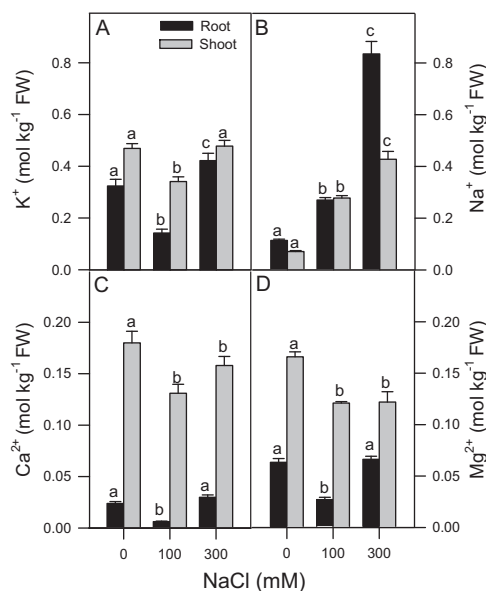
Plant FW was differentially affected under various NaCl concentrations (Fig. 1). Substantial increase in seedling FW was recorded at 100 mM NaCl, however this increase was primarily due to the increase in root weight. Root and shoot fresh weights were decreased substantially when compared to the non-saline control at 300 mM NaCl (Fig. 1). Highest leaf area, shoot height and root/shoot ratio were found at 100 mM NaCl (Fig. 2).

Potassium concentration was substantially higher in plants exposed to 0 and 300 mM NaCl in comparison to those grown at 100 mM NaCl (Fig. 3A).  $\text{Na}^+$  concentration progressively increased with the increase in salinity (Fig. 3B) and it was two-fold higher in roots compared to shoots when treated with 300 mM NaCl. Shoot

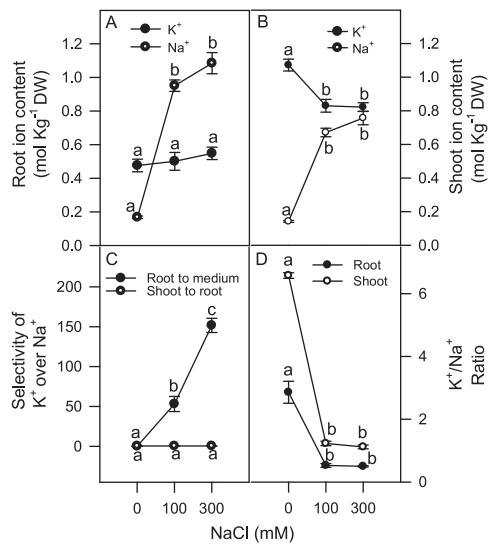


**Fig. 2.** Root/shoot ratio on fresh weight (FW; A), shoot height (B) and leaf area (C) (per plant) of *Phragmites karka* treated with 0, 100 and 300 mM NaCl for 35 days. Values are means  $\pm$  SE. Different letters (LSD) over bars represent significant differences ( $P < 0.05$ ) among NaCl concentrations.

$\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentration was lower in 100 mM NaCl compared to control plant, however there was no change with a further increase in NaCl concentrations (Fig. 3C and D).  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations were significantly lower in roots at 100 mM NaCl than other treatments (Fig. 3C and D). A five-fold increase in root  $\text{Na}^+$  content ( $\text{mol kg}^{-1} \text{DW}$ ) was observed in both organs of plant treated with 100 mM NaCl while no significant difference was found with



**Fig. 3.** Concentration of  $\text{K}^+$  (A),  $\text{Na}^+$  (B),  $\text{Ca}^{2+}$  (C) and  $\text{Mg}^{2+}$  (D) in shoot and root tissues of *Phragmites karka* treated with 0, 100 and 300 mM NaCl for 35 days. Values are means  $\pm$  SE. Different letters (LSD) over bars represent significant differences ( $P < 0.05$ ) among NaCl concentrations.



**Fig. 4.** Ion ( $\text{Na}^+$  and  $\text{K}^+$ ) content in root (A) and shoot (B), selectivity of  $\text{K}^+$  over  $\text{Na}^+$  (C) and  $\text{K}^+/\text{Na}^+$  ratio (D) in tissues of *Phragmites karka* treated with 0, 100 and 300 mM NaCl for 35 days. Values are means  $\pm$  SE. Different letters (LSD) represent significant differences ( $P < 0.05$ ) among NaCl concentration.

a further increase in salinity (Fig. 4A and B). In contrast, shoot  $\text{K}^+$  content significantly decreased in 100 mM NaCl (Fig. 4B). Selectivity of  $\text{K}^+$  over  $\text{Na}^+$  increased in roots with increasing salinity, while no such effect was observed in shoots (Fig. 4C). The  $\text{K}^+/\text{Na}^+$  ratio was higher in shoots than roots and decreased substantially in both plant organs, with the application of salinity (Fig. 4D). Highest values of leaf KUE were found in 300 mM NaCl (Table 1). Leaf N content decreased while C content, C/N ratio and NUE increased under NaCl treatments (Table 1).

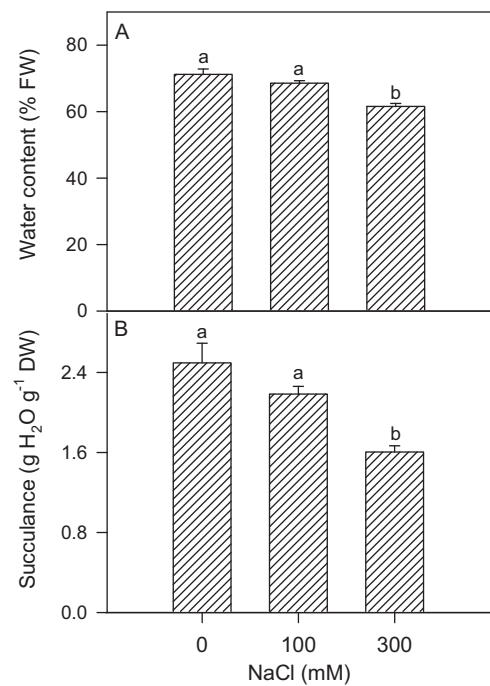
Leaf water potential ( $\psi_w$ ) and osmotic potential ( $\psi_s$ ) were significantly decreased with increase in NaCl (Table 2). Leaf turgor, water content and succulence were significantly lower at 300 mM NaCl compared to plants grown under non-saline condition (Table 2 and Fig. 5).  $\text{Na}^+$  contribution to leaf osmotic potential increased while  $\text{K}^+$  contribution decreased with the induction of salinity and remain unchanged with any further increase in NaCl concentration (Table 2). The contribution of proline and soluble sugars to the total leaf osmotic potential were lowest in 300 mM NaCl (Table 2).

Net photosynthesis was higher in 100 mM NaCl (29%) while a 63% decrease was noted in 300 mM compared to the non-saline control (Table 3). Both leaf stomatal conductance and internal  $\text{CO}_2$  concentration decreased with increase in NaCl. WUE increased significantly with the application of salinity and no difference was found between NaCl concentrations. Respiration and transpiration rates were substantially reduced in 300 mM NaCl (Table 3). Leaf chlorophyll content increased only in 300 mM NaCl (Table 3).

**Table 1**

Change in the content of carbon (C), nitrogen (N), C/N ratio, nitrogen use efficiency (NUE) and potassium use efficiency (KUE) of *Phragmites karka* leaf treated with 0, 100 and 300 mM NaCl for 35 days. Values are means  $\pm$  SE. Means are considered different at  $P < 0.05$  (LSD) when followed by different letters within the parameters measured.

Treatments	NaCl (mM)		
	0	100	300
C (% DW)	36.83 $\pm$ 0.71a	38.55 $\pm$ 0.25b	39.00 $\pm$ 0.13b
N (% DW)	2.93 $\pm$ 0.07a	2.63 $\pm$ 0.09b	2.33 $\pm$ 0.06c
C/N ratio	11.92 $\pm$ 0.52a	14.05 $\pm$ 0.57b	16.32 $\pm$ 0.54c
NUE (g plant DW g <sup>-1</sup> leaf N)	96.22 $\pm$ 9.49a	126.02 $\pm$ 12.22b	144.34 $\pm$ 13.90c
KUE (g mg <sup>-1</sup> )	0.09 $\pm$ 0.02a	0.10 $\pm$ 0.00a	0.12 $\pm$ 0.00b



**Fig. 5.** Leaf water content (A) and leaf succulence (B) of *Phragmites karka* treated with 0, 100 and 300 mM NaCl for 35 days. Values are means  $\pm$  SE. Different letters (LSD) over bars represent significant differences ( $P < 0.05$ ) among NaCl concentrations.

#### 4. Discussion

*P. karka* is a grass distributed in saline marshes and wetlands with considerable potential as a source of biofuel (Abideen et al., 2012), medicinal compounds (Sharma and Pegu, 2011) and dry fodder (Koirala and Jha, 2013). Our data explains the mechanism of salt resistance and provides information regarding optimal biomass production.

Optimum growth of *P. karka* was obtained in 100 mM NaCl while most of the growth parameters showed a decreasing trend in 300 mM. Data available on some of the halophyte grasses (*Aeluropus lagopoides*, *Sporobolus ioclados*, *Urochondra setulosa* and *Halopyrum mucronatum*) indicate optimum growth under non-saline conditions (Gulzar and Khan, 2006; Khan et al., 1999). However, in some of the grasses, growth may be optimized in moderately saline conditions (Flowers and Colmer, 2008). Besides *P. karka*, other halophytic grasses displayed a classical dose-dependent response as in *Phragmites australis* (Gorai et al., 2010), *Phragmites communis* (Gorai et al., 2007), *Spartina maritima* (Naidoo et al., 2012) and *Pennisetum clandestinum* (Muscolo et al., 2003). Salt resistance in some of the closely related ecotypes of *P. australis* is reported to range between 0.14 and 1.2 M NaCl (Achenbach et al., 2013).

Root growth in *P. karka* was stimulated under moderately saline conditions which is also reported for other salt resistant grasses (*Distichlis spicata*, *Sporobolus airoides*, *Cynodon dactylon*, *Zoysia japonica*; Marcum, 1999) and *Puccinellia ciliata* (Teakle et al., 2013). Extensive root growth of *P. karka* could help in the absorption of  $\text{K}^+$  and storage of  $\text{Na}^+$  in the below ground part by preventing  $\text{Na}^+$  loading into the xylem stream up to a threshold salinity concentration (Munns and Tester, 2008). Under high salinity, root growth was reduced, thus limiting net  $\text{Na}^+$  influx and preventing  $\text{Na}^+$  translocation to the shoot (Gorham et al., 1985).

Salt resistance in halophytic grasses is usually associated with their ability to maintain  $\text{K}^+$  homeostasis (Peng et al., 2004), compartmentalize  $\text{Na}^+$  in root (Ahmed et al., 2013), restrict  $\text{Na}^+$  uptake and/or transport from root to shoot (Carden et al., 2003; Teakle

**Table 2**  
Leaf osmotic potential ( $\psi_s$ ), water potential ( $\psi_w$ ) and turgor potential ( $\psi_t$ ) with contributions of  $\text{Na}^+$ ,  $\text{K}^+$ , proline and soluble sugar to osmotic potential in leaves of *Phragmites karka* treated with 0, 100 and 300 mM NaCl for 35 days. Values are means  $\pm$  SE. Means are considered different at  $P < 0.05$  (LSD) when followed by different letters within the parameters measured.

NaCl (mM)	$\psi_s$ (MPa)	$\psi_w$ (MPa)	$\psi_t$ (MPa)	Contribution to $\psi_s$ (%)			
				$\text{Na}^+$	$\text{K}^+$	Proline	Soluble Sugar
0	1.3 $\pm$ 0.2a	0.2 $\pm$ 0.02a	1.0 $\pm$ 0.01a	1.9 $\pm$ 0.1a	25.1 $\pm$ 2.1a	1.5 $\pm$ 0.03a	15.1 $\pm$ 1.4a
100	1.8 $\pm$ 0.6b	0.6 $\pm$ 0.03b	1.2 $\pm$ 0.02a	14.0 $\pm$ 1.0b	17.7 $\pm$ 1.3b	1.6 $\pm$ 0.04a	16.0 $\pm$ 0.4a
300	2.8 $\pm$ 2.5c	2.5 $\pm$ 0.11c	0.3 $\pm$ 0.01b	11.8 $\pm$ 0.4b	19.0 $\pm$ 0.6b	0.6 $\pm$ 0.06b	10.3 $\pm$ 0.5b

**Table 3**  
Gas-exchange parameters ( $A_N$ , photosynthesis;  $G_s$ , stomatal conductance;  $C_i$ , intercellular concentration of  $\text{CO}_2$ ;  $E$ , transpiration rate;  $R$ , respiration), water use efficiency (WUE) and chlorophyll content of *Phragmites karka* treated with 0, 100 and 300 mM NaCl for 35 days. Values are means  $\pm$  SE. Means are considered different at  $P < 0.05$  (LSD) when followed by different letters within the parameters measured.

Parameters	NaCl (mM)		
	0	100	300
$A_N$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	18.40 $\pm$ 1.58a	24.69 $\pm$ 0.75b	11.95 $\pm$ 0.56c
$G_s$ ( $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )	0.28 $\pm$ 0.01a	0.19 $\pm$ 0.01b	0.08 $\pm$ 0.01c
$C_i$ ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	273.01 $\pm$ 7.11a	187.07 $\pm$ 6.50b	123.36 $\pm$ 13.10c
$E$ ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )	5.36 $\pm$ 0.28a	5.58 $\pm$ 0.05a	1.48 $\pm$ 0.17b
WUE ( $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ )	64.34 $\pm$ 3.56a	118.64 $\pm$ 4.05b	148.74 $\pm$ 18.11b
$R$ ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	-4.57 $\pm$ 0.56a	-3.20 $\pm$ 0.70a	-1.39 $\pm$ 0.32b
Chlorophyll content (SPAD relative values)	40.51 $\pm$ 0.56a	43.91 $\pm$ 0.36a	47.47 $\pm$ 0.45b

et al., 2013). *P. karka* appeared to sequester  $\text{Na}^+$  in the root and regulate transport of  $\text{Na}^+$  toward the shoot possibly by using HKT, PM-NHX and V-NHX proteins (Ahmed et al., 2013; Kronzucker et al., 2013). Sodium content was similar in the above-ground part of plant under moderate and high salinity. Under high salinity, transpiration was substantially reduced which may have restricted  $\text{Na}^+$  transfer to the shoot. In addition, re-translocation of  $\text{Na}^+$  from shoot to root might be a strategy in *P. karka* as previously reported in *P. australis* (Gorai et al., 2007; Takahashi et al., 2007; Pagter et al., 2009). However,  $\text{K}^+$  homeostasis was achieved in roots by enhancing its selective absorption. Decrease in shoot  $\text{K}^+$  content may be due to the limited  $\text{K}^+$  transport from root to shoot. Nevertheless, under moderate salinity (100 mM NaCl) shoots maintain  $\text{K}^+/\text{Na}^+$  greater than 1 needed for cellular functioning (Silva et al., 2012) and to prevent salt induced plant death (Shabala, 2009). Decrease in shoot  $\text{K}^+$  may also contribute in the reduction of plasma membrane depolarization (Bonales-Alatorre et al., 2013). *P. karka* exposed to high salinity improve KUE to overcome the effect of limited K content.

*P. karka* considerably lowers the water and osmotic potential of leaves when exposed to increasing NaCl concentration in its growth medium. Halophytic grasses are reported to increase leaf osmolality instead of developing succulence (Rozema and Schat, 2012) and attain osmotic adjustment at moderate salinity level (Koyro, 2006; Gorai et al., 2010). Under high salinity, water content and succulence of *P. karka* leaves were significantly lower in comparison to non-saline treatments. This is possibly due to a reduction in transpiration rate, which resulted in turgor loss. Our data suggest that turgidity plays a more important role than succulence which is in agreement with findings on other monocots (Parida and Das, 2005).

Osmotic adjustment in plants is achieved by accumulating both inorganic and organic solutes (Flowers and Colmer, 2008; Rozema and Schat, 2012). In *P. karka* proline and sugars appear to play little role in osmotic adjustment ( $\leq 15\%$ ). However, they could be involved in other physiological processes such as osmo-protection or free radical scavenging (Natarajan et al., 2012). On the contrary,  $\text{Na}^+$  and  $\text{K}^+$  collectively contributed to about 30% of the osmotic adjustment (twice the amount of organic compounds). Moreover,  $\text{Na}^+$  appeared to replace  $\text{K}^+$  contribution in osmotic adjustment under salinity treatments. This indicates that *P. karka* prefers inexpensive means for osmotic adjustment (Yeo, 1983).

However, other osmotically active solutes like anions, glycinebetaine, etc. could contribute in achieving osmotic balance along with cations (Alikhani et al., 2011).

The rate of gas exchange varies with the intensity of abiotic stresses such as salinity and drought (Geissler et al., 2009). Elevated photosynthesis rate in *P. karka* was observed at moderate rather than higher salinity concentrations and this trend was positively related with plant growth. Higher photosynthesis in plants exposed to moderate salinity might be due to efficient fixation of  $\text{CO}_2$  at lower intracellular  $\text{CO}_2$  concentration. Plant species respond to low soil water potential either through stomatal closure that restrict  $\text{CO}_2$  availability for carboxylation, or by non-stomatal inhibition, caused by damaging effects of salt on photosynthetic machinery (Flexas et al., 2004). Stomatal conductance decreased in *P. karka* under high salinity limiting the  $\text{CO}_2$  supply which caused considerable reduction in photosynthesis. A relatively low level of intracellular  $\text{CO}_2$  concentration observed in *P. karka* plants treated with 300 mM NaCl similar to that reported in *P. australis* (Choi et al., 2005), support our interpretation. Increase in WUE of *P. karka* under high salinity appears to be due to the reduction in transpiration rate rather than  $\text{CO}_2$  assimilation (Pagter et al., 2009). Based on these findings we assume that the reduced availability of water and  $\text{CO}_2$  in leaves were the main causes of reduced photosynthesis at high salinity. Koyro (2006) suggested that reduction in stomatal conductance represent adaptive mechanisms to prevent increase in salt load of leaves which could reach to toxic level if transpiration is not checked.

Leaf C/N ratio and NUE in *P. karka* correlated positively with salinity. The increasing salinity leads to a slight increase in C content while N content decreased causing consistently higher C/N ratio. This effect could be ecologically important particularly for nutrient deficient soils, since nitrogen is usually considered to be a limiting factor for plant growth performance under natural conditions (Pakeman and Lee, 1991). The slight increase in C/N ratio under moderate salinity indicates that carbon fixation and nitrate reduction are not tightly linked, but energy flow is preferentially directed toward  $\text{CO}_2$  fixation.

In conclusion, growth of *P. karka* increased at moderate salinity and particularly higher root biomass which help in accumulating a large quantity of  $\text{Na}^+$  leading to its exclusion from leaves while maintaining adequate levels of shoot  $\text{K}^+$  through higher selectivity.

**Table 4**

Change in eco-physiological parameters of *P. karka* after 35 day exposure to 100 and 300 mM NaCl. The direction of arrow shows the change in comparison with the non-saline treatment, and number of arrow shows the corresponding higher response at  $P < 0.05$ .

Parameters	NaCl (mM)	
	100	300
Plant growth	↑	↓
Root/shoot growth	↑	—
Water relations	—	↓
Na <sup>+</sup>	↑	↑↑
K <sup>+</sup> /Na <sup>+</sup> (shoot)	↓	↓
K <sup>+</sup> /Na <sup>+</sup> (root)	↓	↓
K <sup>+</sup> selectivity (root to medium)	↑	↑↑
K <sup>+</sup> selectivity (shoot to root)	—	—
Photosynthesis	↑	↓
Respiration	—	↓
Stomatal conductance	↓	↓↓
Transpiration rate	—	↓
WUE	↑	↑
KUE	—	↑
C/N	↑	↑↑
NUE	↑	↑↑

In addition favorable water relations, effective osmotic adjustment using primarily cheap osmoticum contributed to increased photosynthesis and higher biomass production (Table 4). Growth inhibition under higher salinity could be attributed to limited salt excluding ability, low leaf tissue hydration and turgor loss, leading to stomatal closure and decreased CO<sub>2</sub> absorption (Table 4).

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