

Growth patterns of *Phragmites karka* under saline conditions depend on the bulk elastic modulus*

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Abstract. Salt stress is known to hamper steady-state water flow, which may reduce plant growth. This research was aimed to study the roles of leaf turgor, osmotic adjustment and cell wall elasticity under saline conditions which may reduce biomass production in *Phragmites karka* (Retz.) Trin, ex. Steud. (a marsh grass). Plants were grown in 0, 100 and 300 mM NaCl and harvested on 3, 7, 15 and 30 days to observe periodic changes in growth and water relations. Leaf number, relative growth rate, and relative elongation rates were higher in the non-saline control than in the plants grown under saline conditions. Plants showed a rapid decline in leaf growth rate (7–15 days) in 300 mM NaCl compared with a delayed response (15–30 days) in 100 mM NaCl. Leaf water potential decreased with increases in salinity after the third day of exposure whereas osmotic potential decreased after the fifteenth day. Low leaf turgor (Ψ_p) on the third day indicated an initial phase of osmotic stress under saline conditions. Plants maintained higher Ψ_p in 0 and 100 mM than in 300 mM NaCl. Differences between mid-day and pre-dawn water potential and water saturation deficit were higher in 300 mM NaCl than with other treatments. Water potential and hydraulic capacitance at turgor loss point decreased whereas bulk elastic modulus increased in 300 mM NaCl. Maintenance of turgor and growth at 100 mM NaCl could be related to efficient osmotic adjustment (use of K^+ and Cl^-), higher WUE_i , and lower bulk elasticity whereas poor growth at 300 mM NaCl may have been a consequence of low turgor, decreased cell hydraulic capacitance and higher bulk elastic modulus.

Additional keywords: cell wall rigidity, diurnal water relation, leaf turgor, marshy grass, membrane rigidity, pressure volume curve.

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Introduction

Low water availability caused by drought, salinity, or other abiotic factors usually limits plant growth and development (Mahajan and Tuteja 2005; Munns and Tester 2008; Álvarez *et al.* 2012). Among these abiotic stresses, salinity has become an increasingly serious problem, which restricts yield on almost 50% of irrigated land globally (Ruan *et al.* 2010). Depleting fresh water resources and increasing salt-affected areas are major hurdles for crop cultivation (Ruan *et al.* 2010). Hence, growing

crop plants as alternate sources of energy (e.g. biofuel) would further increase pressure on cultivated lands (Abideen *et al.* 2012). In view of the abovementioned problems, domestication of non-conventional crops on saline waste lands seems a viable alternative (Reddy *et al.* 2008; Rozema and Flowers 2008; Abideen *et al.* 2012).

Halophytes are capable of maintaining high water-use efficiency levels (Chaves *et al.* 2009; Riccardi *et al.* 2014) and better yields when grown in saline habitats than non-halophytes

Abbreviations: Ψ_w , Leaf water potential; Ψ_π , Leaf osmotic potential; Ψ_p , Leaf turgor potential; ΨW_0 , Water potential at full turgor; ΨS_0 , Osmotic potential at full turgor; ΨP_0 , Turgor potential (PVC derived); ΨW_{TLP} , Water potential at turgor loss point; RWC_0 , Relative water content at full turgor; RWC_{TLP} , Relative water content at turgor loss point; C_{FT} , Hydraulic capacitance at full turgor; C_{TLP} , Hydraulic capacitance at turgor loss point; ϵ , Bulk elastic modulus; LTD, Leaf tissue density; PVC, Pressure volume curve; OA_t , Total osmotic adjustment; AOA, Active osmotic adjustment; POA, Passive osmotic adjustment; FW, Fresh weight; RGR_{leaf} , Relative growth rate of leaf; RER_{leaf} , Relative elongation rate of leaf; SUC, Succulence; WSD, Water saturation deficit; WUE_i , Instantaneous water-use efficiency.

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(Khan and Ansari 2008; Glenn et al. 2013; Ventura and Sagi 2013). In view of an increasing world food demand, the growth dynamics of conventional crops with changing soil water potential have been extensively discussed in some species (Romero-Aranda et al. 2001; Fernández-García et al. 2004; Netondo et al. 2004) but limited information exists on water relation of giant halophytic grasses.

Plants living in salt-stressed environments respond differently to soil water potential than those growing under non-saline conditions. One of the important mechanisms is osmotic adjustment, the accumulation of solutes that could help in achieving osmotic balance and water uptake for maintaining growth (Flowers and Colmer 2008). In some plants, particularly grasses, synthesising organic osmolytes may compromise growth (Roy and Chakraborty 2014). Changes in growth are not only related to physiological attributes but, they are also linked with morphological and anatomical characters (Bose et al. 2015; Flowers et al. 2015). Changes in turgor due to cell wall elasticity, increased succulence and tissue density represent growth modulation (Hoffmann and Poorter 2002). Some plants alter bulk elastic modulus, increase leaf thickness and tissue density whereas others change their relative water content to maintain turgor (Gullo and Salleo 1988; Villar-Salvador 2004). Plants with highly elastic tissues maintain higher amounts of water at full saturation, and hence at the turgor loss point they may have larger hydraulic capacitance with respect to the cell volume (Lambers et al. 2008; Bartlett et al. 2012). However, rigid walls would decrease water potential with small water loss (Navarro et al. 2007). Parameters such as osmotic potential at full turgor (Ψ_{S0}), water potential at turgor loss point ($\Psi_{W_{TLP}}$) and bulk elastic modulus (ϵ) derived from 'Pressure volume curve' (PVC) are commonly used to understand the dynamics of leaf water relations to osmotic adjustment as well as plant growth (Bartlett et al. 2012). In addition, the concentrations of calcium, which is important as a signalling molecule and structural component of the cell wall (Hepler and Winship 2010; Bickerton and Pittman 2012; Parvin et al. 2016), may increase in plant tissues under saline conditions, and therefore it is important to understand the link between calcium content and cell wall elasticity.

Phragmites karka (Retz.) Trin, ex. Steud, is a perennial halophytic grass distributed on saline marshy habitats around Karachi (Zehra and Khan 2007). This species is also present in the native flora of Australia (<https://florabase.dpaw.wa.gov.au/browse/profile/556>, accessed 23 March 2018). *P. karka* produces large amounts of biomass and, like other grasses such as *Arundo donax*, *Phragmites australis*, and *Miscanthus giganteus* is considered to be a potential candidate for biofuels because of its suitable ligno-cellulosic composition (Sims et al. 2006; Barney and DiTomaso 2008; Abideen et al. 2012; Cotana et al. 2015). It was reported that *P. karka* maintained growth and water relations under moderate salinity (100 mM), whereas at 300 mM NaCl low leaf hydration and turgor loss reduced plant growth (Abideen et al. 2014). However, it remains unclear whether water deprivation or increasing salt loads in the roots of *P. karka* is the major cause of turgor loss, and little information is available about the impact of salinity on plant water relations, and particularly regarding structural modification in this species.

The present work is based on the hypothesis that the growth response of *P. karka* under salinity depends on the bulk elastic modulus. This study was carried out to determine how the duration of salt exposure and diurnal changes in water relations interact with bulk elastic modulus (ϵ) for controlling growth. The present study also describes a link among growth, water relation attributes and cell wall rigidity of *P. karka* in saline condition that has not been described in previous research on giant grasses.

Materials and methods

Seeds of *Phragmites karka* were collected from marshy habitats of Karachi University, Pakistan (24°55'3"N and 67°6'19"E) in February 2014. Seeds were sown in plastic trays containing sandy soil plus manure and irrigated with water for germination. Five-week-old seedlings of similar size (length 6–8 cm, three-leaf stage) were transplanted into plastic pots (6 cubic litres; three individuals per pot) containing sand and loam (2:1) and sub-irrigated with 1/2 strength Hoagland nutrient solution (modified after Epstein 1972). Plants were grown in a netted greenhouse under ambient environmental conditions (temperature: $37 \pm 1^\circ\text{C}$; relative humidity: $50 \pm 5\%$; light intensity: $450 \pm 10 \mu\text{mol m}^{-2} \text{s}^{-1}$). Salinity treatments were started after 30 days of seedling acclimatisation. Plants were divided into three salt treatment groups (0, 100, 300 mM NaCl) and five sub-groups (0, 3, 7, 15, 30 days). In order to avoid osmotic shock, NaCl was applied gradually with an increase of 50 mM per day in the nutrient solution. Treatment solution was completely replaced at weekly intervals until the end of the experiment.

Growth parameters

Growth parameters [fresh weight (FW) and leaf number] were determined at 0, 3, 7, 15, 30 days of salt treatment. Relative growth rate of leaf (RGR_{leaf}) was estimated by using the formula of Hoffmann and Poorter (2002):

$$\text{RGR}_{\text{leaf}} (\text{g g}^{-1} \text{ day}^{-1}) = \ln W_2 - \ln W_1 / t_2 - t_1$$

where, W_1 and W_2 are the initial and final dry weight (g), whereas t_1 and t_2 are the initial and final time (day) from the start of salinity treatments.

Relative leaf elongation rate was calculated using the formula (Muller et al. 2001):

$$\begin{aligned} \text{Relative leaf elongation rate (cm cm}^{-1} \text{ day}^{-1}) \\ = \ln L_2 - \ln L_1 / t_2 - t_1 \end{aligned}$$

where L_1 and L_2 are the initial and final length (cm), whereas t_1 and t_2 are the initial and final time (day) from the start of salinity treatments.

Leaf area was calculated using ImageJ software version 1.45 (<https://imagej.en.softonic.com/download>, accessed 23 March 2018). Leaf sclerophylly indices [succulence (SUC), leaf tissue density (LTD), and water saturation deficit (WSD)] were calculated using the following formulae (Pujol et al. 2001; Denaxa et al. 2012):

$$\text{WSD}(\%) = (\text{TW} - \text{FW}) / (\text{TW} - \text{DW}) \times 100$$

$$\text{SUC} (\text{g H}_2\text{O g}^{-1} \text{ DW}) = \text{FW} - \text{DW} / \text{DW}$$

$$\text{LTD} (\text{g g}^{-1}) = (\text{DW} / \text{FW}) \times 100$$

where, FW, DW and TW are: leaf fresh weight, dry weight and turgid weight, respectively.

Water relation parameters

Water potential and the osmotic potential of leaves were determined on 3, 7, 15 and 30 days after the highest salinity (300 mM NaCl) had been reached. Water potential of 5-mm disks of fully expanded young leaves was determined using a C-52 sample chamber connected to a Wescor HR-33T, thermocouple psychrometer (Wescor, Inc., Logan, UT, USA). Sap osmolality of the same leaf was determined using a Vapor pressure Osmometer (Vapro-5520, Wescor, Inc.). The osmotic contribution of ions (Na^+ , K^+ , and Cl^-) and soluble sugar were calculated using the van't Hoff equation (Guerrier 1996). Transpiration rate and instantaneous water-use efficiency (WUE_i ; net photosynthesis to transpiration ratio) were calculated using Li-COR 6400 instrument (6400XT, Li-Cor Inc., Lincoln, NE, USA).

Calcium content

Calcium (Ca^{+2}) was determined according to Abogadallah (2010). A hot-water extract was prepared from finely ground dried plant material in distilled water at 100°C for 2 h. This filtrate was used to determine leaf Ca^{+2} by atomic absorption spectrometry (AA-700; Perkin Elmer, Santa Clara, CA, USA).

Diurnal water relations

Leaf water and osmotic potentials were determined at pre-dawn and noon to observe changes in diurnal water relations of plant after 15 days of salinity treatments.

Pressure volume curves (PVC)

Fully expanded young leaves were selected on the fifteenth day of salinity treatments for PVC measurements, as leaf hydraulic properties achieve a steady-state after about 2 weeks of salt treatment (Serrano *et al.* 2005). Leaves from the second node were hydrated overnight by covering with plastic bags. PVC were obtained using a Wescor HR-33T, thermocouple psychrometer (Wescor, Inc.) with a C-52 sample chamber (Ogburn and Edwards 2009). The entire sample (leaf and their excised 5-mm Ø disc) was weighed immediately after the leaf was detached from the mother plant. The leaf disc was quickly sealed inside the sample chamber (C-52) to minimise air exposure of the cut surface to avoid any discrepancies in water potential (Walker *et al.* 1984). Samples were allowed to equilibrate at room temperature (29°C). Between measurements, a leaf disc was removed from the sample chamber and allowed to dry for 1–20 min (depending on the hydration state of the leaf tissue). Samples were re-weighed and transferred to a sample chamber for the subsequent water potential reading. This process was repeated 8–10 times per leaf sample to determine the PVC. The inverse of water potential ($1/\Psi_w$) was plotted against relative water content to generate the PVC. A line was drawn as an inflection point in the linear portion of the curve, to determine the turgor loss point (where water and osmotic potentials becomes equal) (Tyree and Hammel 1972). The following water relation parameters were derived from the PVC: osmotic potential at full turgor (Ψ_{S_0}), water potential at turgor loss point ($\Psi_{W_{TLP}}$), relative water content at full

saturation (RWC_0) and turgor loss point (RWC_{TLP}), bulk elastic modulus (ϵ), capacitance at full turgor (C_{FT}) and capacitance at turgor loss point (C_{TLP}) (Tyree and Hammel 1972; Lenz *et al.* 2006; Bartlett *et al.* 2012). Total osmotic adjustment (OA_t) was calculated by subtracting midday Ψ_π in control (0 mM) from those of the saline treatments (100 and 300 mM NaCl) (Boussadia *et al.* 2013). Active osmotic adjustment (AOA) was calculated as the difference in Ψ_{S_0} between control and saline treatments (Girma and Krieg 1992). The contribution of passive osmotic adjustment (POA) to OA_t was calculated using the following formula (Boussadia *et al.* 2013):

$$\text{POA} = \text{OA} - \text{AOA}$$

Statistical analyses

Statistical analysis was conducted using SPSS version 16.0 for windows (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was used to identify significant effects of NaCl concentration and duration of salinity treatment on growth and water relation parameters at $P < 0.05$. Bonferroni and paired sample *t*-test were used to compare individual means. Data in the form of means and standard errors were used to construct graphs using Sigmaplot for Windows version 11.0 (Systat Software, San Jose, CA, USA).

Results

A two-way ANOVA showed a significant effect for salinity (S) ($P < 0.001$, $F = 23.30$), time of exposure (T) to salinity ($P < 0.001$, $F = 36.57$) as well as their interactions (S \times T) ($P < 0.001$, $F = 14.02$) on leaf fresh weight of *P. karka* (Fig. 1a). Similar results were observed with salinity (S) ($P < 0.001$, $F = 64.34$), time (T) ($P < 0.001$, $F = 65.31$) and their interactions (S \times T) ($P < 0.001$, $F = 20.86$) on total plant fresh weight (Fig. 1b). During the initial (15) days of salt exposure both plant and leaf tissue maintained higher fresh weights in 0 and 100 mM NaCl than in 300 mM NaCl (Fig. 1a, b). However, at the end of the experiment (thirtieth day), FW was 2-fold lower in 100 and 4-fold lower in 300 mM NaCl compared with control (Fig. 1a, b). The number of leaves per plant was also similar during 15 days of salinity treatments after which a considerable increase in leaf number at 0 and 100 mM NaCl was observed (Fig. 2a). Leaf senescence was initiated on the seventh day in 300 mM NaCl and the fifteenth day in 0 and 100 mM NaCl treatments. By the end of the experiment, ~85% of leaves were dry (yellow) in 300 mM and only 5% in 100 mM NaCl (Fig. 2b). Higher salinity (300 mM NaCl) significantly ($P < 0.001$) decreased RGR_{leaf} and RER_{leaf} compared with 0 and 100 mM NaCl (Fig. 3a, b). RER_{leaf} was higher whereas RGR_{leaf} was lower during the early phase (15 days) of the experiment in all treatments compared with the later (30 days) phase (Fig. 3a, b).

Leaf sclerophylly indices indicated that there was no change in LTD and SUC under saline conditions, but leaf area decreased and WSD increased in 300 mM NaCl in comparison to other treatments (Table 1). Calculated WUE_i significantly increased ($P < 0.05$) in *P. karka* under salinity treatments whereas transpiration decreased more in 300 mM NaCl than 100 mM NaCl (Table 1). Leaf calcium was unchanged in 100 mM NaCl whereas a significant ($P < 0.05$) increase was

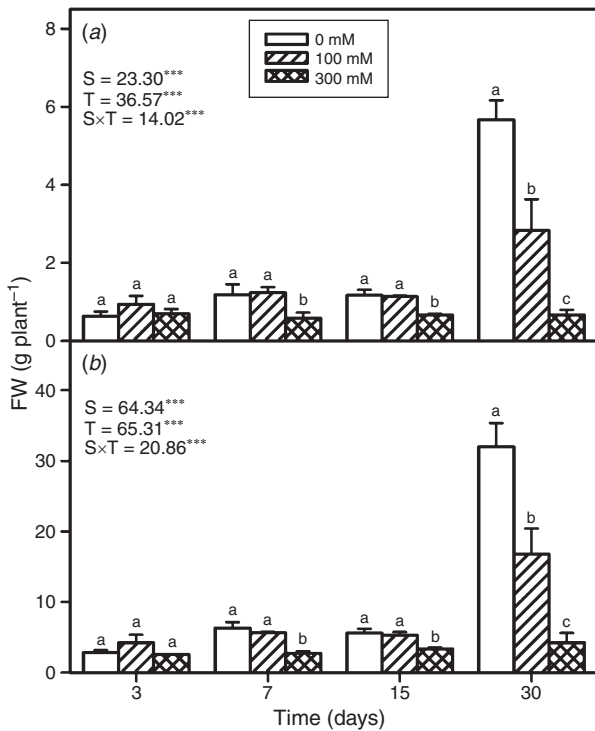


Fig. 1. (a) Fresh weight (FW) of *Phragmites karka* leaves and (b) total plant after NaCl treatment (0, 100 and 300 mM) for different time periods (3, 7, 15 and 30 days). *F* and *P*-values were obtained from ANOVA, where *S* (NaCl treatment), *T* (Time period) and Symbols represent significant levels (**P* < 0.05; ***P* < 0.01; ****P* < 0.001). Values among salinity treatments with similar Bonferroni letters were not significantly different at *P* < 0.05.

found in 300 mM NaCl as compared with the non-saline control (Table 1).

Leaf Ψ_w decreased with increases in NaCl concentration and was more prominent after 7 days of salinity treatment (Fig. 4a). Leaf Ψ_π started to decrease from the seventh day in 300 mM and fifteenth day in 100 mM NaCl, whereas no further reduction was observed between 15 and 30 days of salinity treatment (Fig. 4b). Salinity treatments reduced Ψ_p on the third day; however, no difference in Ψ_p was found between 0 and 100 mM NaCl after 1 week. Moreover, Ψ_p of 300 mM NaCl treated plants significantly increased (*P* < 0.01, *F* = 26.13) after the third day, although it was still lower than the control and 100 mM NaCl treatments (Fig. 4c).

Little variation occurred in water relations between 15 and 30 days, so data on the diurnal pattern with detailed PVC analysis were taken on the fifteenth day only. Pre-dawn leaf Ψ_w and Ψ_π were substantially higher than mid-day values and this difference was more pronounced in 300 mM NaCl (Fig. 5a, b). A paired sample *t*-test indicated that mid-day leaf Ψ_p dropped more significantly (*P* < 0.01) in 300 mM NaCl than in any other treatment (Fig. 5c).

Leaf Ψ_w progressively decreased with increases in salinity whereas Ψ_s decreased under saline conditions with similar values in 100 and 300 mM NaCl (Table 2). Leaf Ψ_p was higher in 100 mM NaCl than other treatments (Table 2).

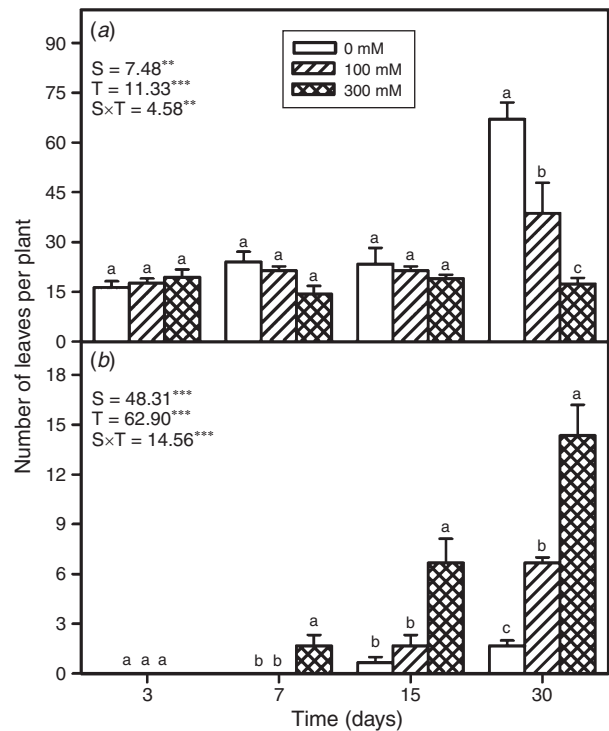


Fig. 2. (a) Number of *Phragmites karka* total leaves and (b) dry leaves after NaCl treatment (0, 100 and 300 mM) for different time periods (3, 7, 15 and 30 days). *F* and *P*-values were obtained from ANOVA, where *S* (NaCl treatment), *T* (Time period), and Symbols represent significant levels (**P* < 0.05; ***P* < 0.01; ****P* < 0.001). Values among salinity treatments with similar Bonferroni letters were not significantly different at *P* < 0.05.

A decrease in Ψ_{wTLP} was observed with increases in salinity (Fig. 6, Table 2). Leaf RWC_0 was similar at 0 and 100 mM and decreased at 300 mM NaCl, whereas RWC_{TLP} in non-saline condition was comparable with that at 300 mM NaCl but was increased in 100 mM NaCl. Differences in relative water content (ΔRWC) between RWC_0 and RWC_{TLP} were higher in 0 and 100 mM NaCl (~50% and 32% respectively) and lower in 300 mM NaCl (~26%) (Fig. 6, Table 2). Hydraulic capacitance decreased at full turgor (C_{FT}) in 100 mM, while at turgor loss point (C_{TLP}) in 300 mM NaCl. There was a difference of $\sim 2.5 \text{ mol m}^{-2} \text{ MPa}^{-1}$ between C_{FT} and C_{TLP} in 0 and 100 mM NaCl, whereas this difference was $0.13 \text{ mol m}^{-2} \text{ MPa}^{-1}$ in 300 mM NaCl (Table 2). Bulk elastic modulus (ϵ) was similar at 0 and 100 mM and increased in 300 mM NaCl (Table 2). The contribution of ions (K^+ and Cl^-) to osmotic adjustment was 55% in saline-treated plants whereas the rest was attributed to organic osmolytes (Table 3). No difference was found in OA_t between 100 and 300 mM NaCl. Plants showed active osmotic adjustment (AOA) in both NaCl treatment whereas passive osmotic adjustment (POA) only at 300 mM NaCl (Table 3).

Pearson analysis showed significant negative correlation of ϵ with Ψ_{wTLP} , leaf FW and plant FW (Table 4). In addition, Ψ_s was positively correlated with Ψ_{wTLP} , whereas Ψ_{wTLP} was positively correlated with leaf and plant FW (Table 4).

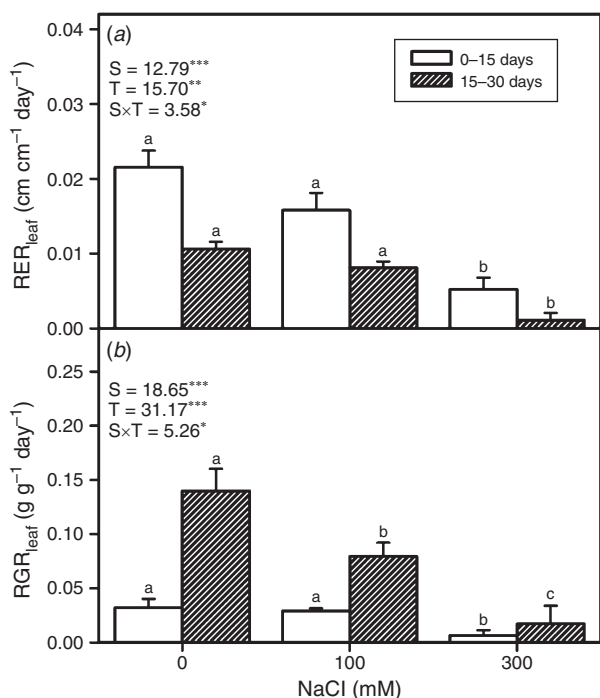


Fig. 3. Relative leaf elongation rate (RER_{leaf}) and relative growth rate (RGR_{leaf}) of *Phragmites karka* leaves after different duration (0–15 and 15–30 days) of NaCl treatment (0, 100 and 300 mM). *F* and *P*-values were obtained from ANOVA, where *S* (NaCl treatment), *T* (Time period), and Symbols represent significant levels (**P* < 0.05; ***P* < 0.01; ****P* < 0.001). Values among salinity treatments with similar Bonferroni letters were not significantly different at *P* < 0.05.

Table 1. Changes in calcium content, sclerophylly indices and water relation parameters of leaves under salinity

Leaf area (LA), calcium content (Ca²⁺), leaf tissue density (LTD), succulence (SUC), water saturation deficit (WSD), instantaneous water-use efficiency (WUE_i) and transpiration (E) of *Phragmites karka* treated with 0, 100 and 300 mM NaCl for 15 days. Values among salinity treatments followed by similar Bonferroni letter are not significantly different at *P* < 0.05

Parameters	NaCl (mM)		
	0	100	300
LA (cm ² plant ⁻¹)	67.4 ± 7.5a	72.5 ± 6.8a	38.8 ± 0.6b
Ca ²⁺ (mg g ⁻¹ DW)	2.8 ± 0.3b	3.2 ± 0.3ab	4.4 ± 0.1a
LTD (g g ⁻¹)	0.3 ± 0.0a	0.3 ± 0.0a	0.3 ± 0.0a
SUC (g g ⁻¹ DW)	2.6 ± 0.3a	2.7 ± 0.0a	2.8 ± 0.1a
WSD (%)	3.4 ± 0.3b	5.8 ± 1.2b	28.0 ± 2.8a
WUE _i (μmol CO ₂ mmol ⁻¹ H ₂ O)	36.1 ± 2.2b	86.6 ± 1.1a	80.7 ± 1.3a
E (mmol m ⁻² s ⁻¹)	5.9 ± 1.0a	3.1 ± 0.5b	1.6 ± 0.4c

Discussion

Salinity lowers soil water potential, which may restrict water supply to the plants (Dulai *et al.* 2011). Morpho-metric changes such as leaf elongation rate, leaf development, reduced transpiration, and stomatal closure disrupt plant water balance and growth under stress (Rasheed *et al.* 2016). In this study the response in growth of *Phragmites karka* to periodic changes in water relations under NaCl treatments was determined.

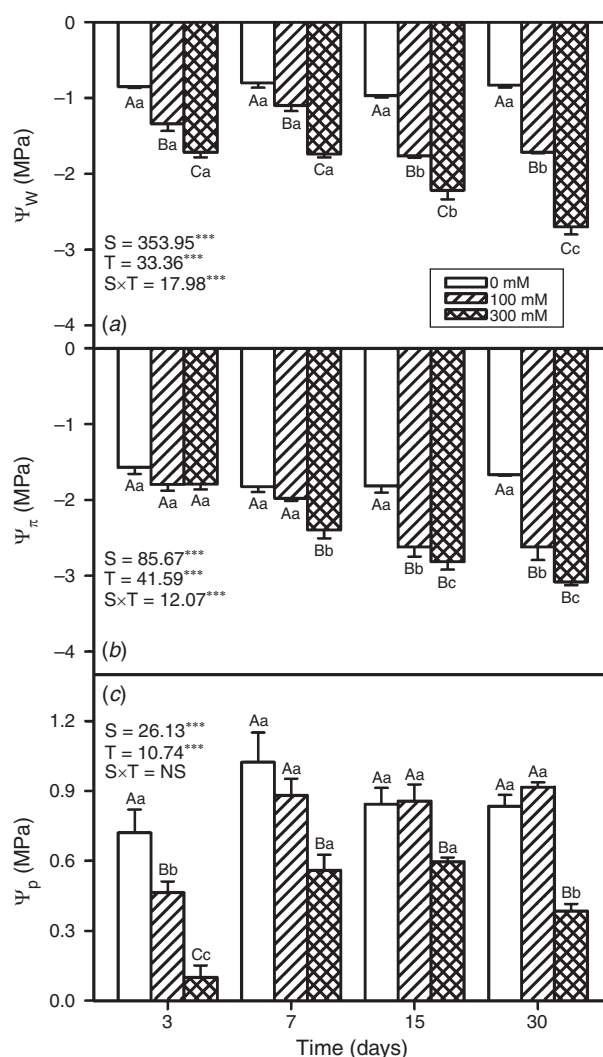


Fig. 4. Water potential (Ψ_w; a), osmotic potential (Ψ_π; b) and turgor potential (Ψ_p; c) of *Phragmites karka* after NaCl treatment (0, 100 and 300 mM) for different time periods (3, 7, 15 and 30 days). *F* and *P*-values were obtained from ANOVA, where *S* (NaCl treatment), *T* (Time period), and Symbols represent significant levels (**P* < 0.05; ***P* < 0.01; ****P* < 0.001; NS, non-significant). Similar capital letters among salinity treatments and small letters within each salinity treatment are not significantly different (*P* < 0.05) from each other, Bonferroni test.

Growth responses and biomass production of *Phragmites karka* were similar at 0 and 100 mM until the fifteenth day compared with 300 mM NaCl. However, by the end of the experiment (30 days) optimum plant growth (fresh weight and leaf number) occurred in the non-saline control, which is a common feature in some marsh grasses such as *Aeluropus lagopoides*, *Sporobolus ioclados*, and *Urochondra setulosa* (Gulzar and Khan 2008). Abideen *et al.* (2014) reported better plant growth of *P. karka* at 100 mM NaCl (moderate salinity) compared with growth under non-saline conditions in a quick check system (light intensity: 250 μmol m⁻² s⁻¹; temperature: 25°C), which is similar to other species such as, *Spartina alterniflora* and *Spartina maritima* (Ma *et al.* 2011;

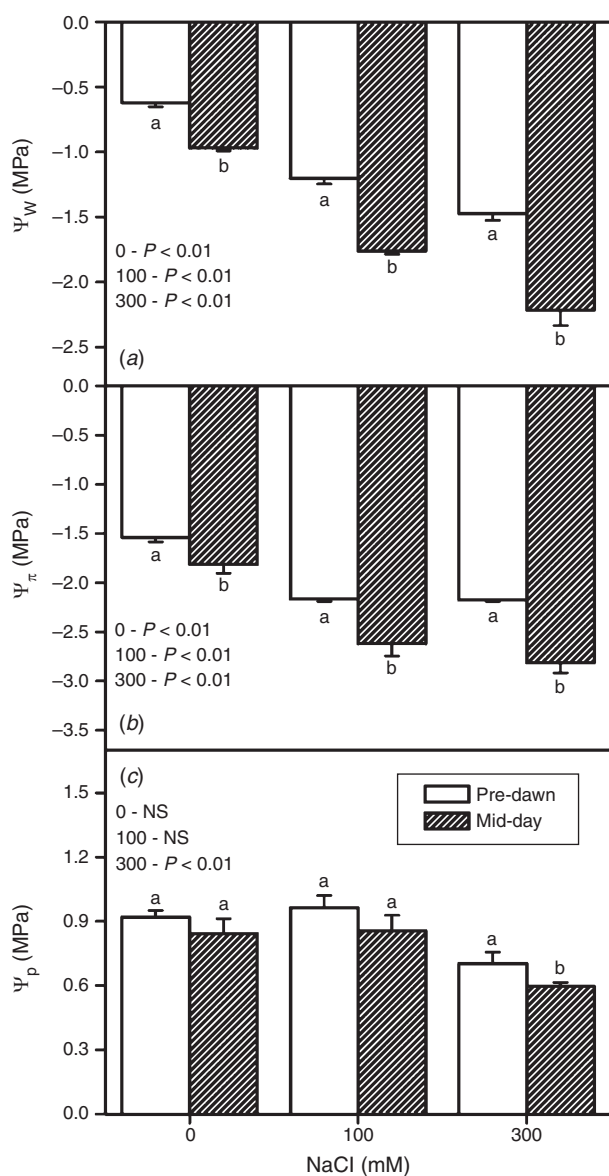


Fig. 5. Pre-dawn and mid-day water potential (Ψ_w ; a), osmotic potential (Ψ_π ; b) and turgor potential (Ψ_p ; c) of *Phragmites karka* treated with different concentrations of NaCl (0, 100 and 300 mM) for 15 days. *P*-values represent the degree of significance in *t*-test analysis. Values within each salinity treatments with similar letters are not significantly different according to the *t*-test.

Naidoo *et al.* 2012). In this experiment, the reduced growth at 100 mM NaCl could be attributed to high light intensity ($\sim 500 \mu\text{mol m}^{-2} \text{s}^{-1}$) and ambient temperature (37°C). In the early vegetative phase (15 days), *P. karka* appeared to invest in leaf elongation rather than in new leaf development, (which demands high energy consumption) and hence maintained fresh weights at 100 mM NaCl that were similar to the non-saline control. However, with advancing age (30 days), plants appeared to invest in new leaf development irrespective of the salinity treatments. Moreover, biomass reduction in moderately saline condition could also be linked with energy requiring

Table 2. Changes in leaf parameters related to pressure volume curve Water potential at full turgor (Ψ_{W_0}), osmotic potential at full turgor (Ψ_{S_0}), turgor potential (Ψ_{P_0}), water potential at turgor loss point ($\Psi_{W_{TLP}}$), relative water content at full turgor (RWC_0), relative water content at turgor loss point (RWC_{TLP}), hydraulic capacitance at full turgor (C_{FT}), hydraulic capacitance at turgor loss point (C_{TLP}) and bulk elastic modulus (ϵ) of *Phragmites karka* treated with 0, 100 and 300 mM NaCl for 15 days. Values among salinity treatments followed by similar Bonferroni letters are not significantly different at $P < 0.05$

Parameters	NaCl (mM)		
	0	100	300
Ψ_{W_0} (-MPa)	$0.6 \pm 0.0a$	$1.1 \pm 0.0b$	$1.5 \pm 0.0c$
Ψ_{S_0} (-MPa)	$1.2 \pm 0.0a$	$2.0 \pm 0.1b$	$2.0 \pm 0.0b$
Ψ_{P_0} (MPa)	$0.6 \pm 0.1b$	$0.9 \pm 0.0a$	$0.5 \pm 0.1b$
$\Psi_{W_{TLP}}$ (-MPa)	$1.3 \pm 0.0a$	$2.1 \pm 0.1b$	$2.5 \pm 0.0c$
RWC_0 (%)	$96.5 \pm 0.3a$	$94.3 \pm 2.9a$	$71.5 \pm 3.0b$
RWC_{TLP} (%)	$44.9 \pm 4.3b$	$63.0 \pm 3.6a$	$44.8 \pm 3.5b$
C_{FT} ($\text{mol m}^{-2} \text{MPa}^{-1}$)	$0.4 \pm 0.1a$	$0.2 \pm 0.0b$	$0.2 \pm 0.0ab$
C_{TLP} ($\text{mol m}^{-2} \text{MPa}^{-1}$)	$3.0 \pm 0.6a$	$2.6 \pm 0.5a$	$0.3 \pm 0.0b$
ϵ (MPa)	$2.4 \pm 0.4b$	$2.6 \pm 0.4b$	$5.3 \pm 0.5a$

processes such as nutrient homeostasis, ROS quenching, and osmotic adjustment (Asrar *et al.* 2017).

Phragmites karka displayed a classical time- and dose-dependent salinity response in growth and water relations, which is similar to those observed in other halophytic grasses (Liu *et al.* 2011; Ahmed *et al.* 2013). Plants regulate their biomass (plant FW, leaf FW, RGR_{leaf}) at 100 mM NaCl with the help of higher relative water content and better WUE_i (Nerd and Pasternak 1992; Riccardi *et al.* 2014). Growth reduction of *P. karka* in 300 mM NaCl within 3–7 days seems to be due to osmotic rather than ionic effects of salinity, which may be related to leaf turgor loss and tissue dehydration (Abideen *et al.* 2014). However, growth reduction (RGR_{leaf} , RER_{leaf}) with increased number of dead leaves during long-term exposure (30 days) of 300 mM NaCl is probably due to both osmotic and ionic effects of salinity (Munns and Termaat 1986; Wang and Nii 2000; Munns and Tester 2008).

Calcium plays an important role in plant growth under saline conditions because of its involvement in signalling cascades and structural modifications (Hepler and Winship 2010; Bickerton and Pittman 2012; Parvin *et al.* 2016). In the present study, a considerable increase in Ca^{+2} content at 300 mM NaCl may be linked with high cell wall rigidity of *P. karka*. It appears that Ca^{+2} was involved in the hardening of cell walls via complexes of Ca-pectate (Cosgrove 2005; Hepler and Winship 2010), which may result in an inhibition of cell elongation and leaf area. Calcium content at 100 mM NaCl was similar to the non-saline control, which is concomitant with unaltered bulk elastic modulus. However, the involvement of Ca^{+2} signalling in stomatal opening (Webb *et al.* 1996) and aquaporin function (Cabañero *et al.* 2006) in moderate salinity requires further investigation.

Leaf osmotic potential (Ψ_π) was similar among salinity treatments on the third day resulting in lower leaf turgor, which indicates that plants faced difficulty in obtaining water due to the osmotic effect of salinity (Munns and Tester 2008). However, a prominent decrease in Ψ_π at 100 mM NaCl after 15 days indicates better osmotic adjustment. A rapid decrease

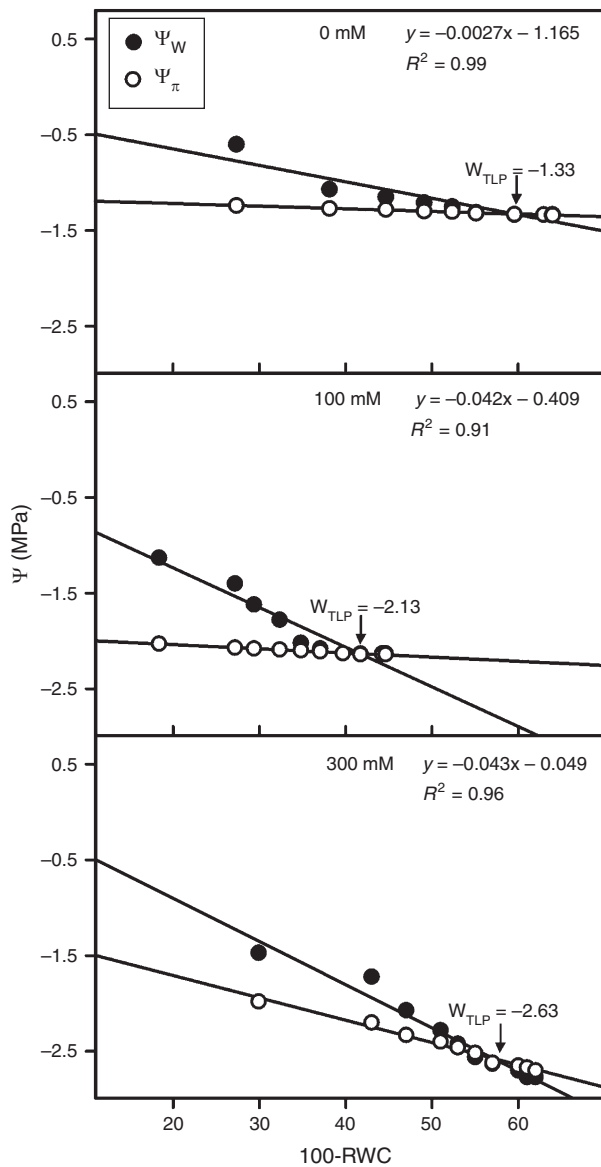


Fig. 6. Pressure volume curve of *Phragmites karka* treated with different concentrations of NaCl (0, 100 and 300mM) for 15 days. The sign of Ψ represents potential of water (Ψ_w), solute (Ψ_π) and water potential at the turgor loss point (W_{TLP}), and relative water content (RWC).

in leaf Ψ_π at 100 mM NaCl indicates that the osmo-regulator strategy of *Phragmites karka* may be similar to *P. australis* (Vasquez *et al.* 2006; Gorai *et al.* 2011) and several other grasses such as *Sporobolus tremulus*: Moinuddin *et al.* (2014); *Andropogon greenwayi*: Hamilton *et al.* (2001). This strategy enables osmotic adjustment in plants where frequent changes in soil salinity are not common (Adam 1993). Most of the halophytic species use Na^+ and Cl^- as cheap osmoticum because the synthesis of organic osmolytes requires high energy (Wang *et al.* 2004). The osmotic contribution of inorganic content was ~50% in *P. karka* under salinity. Among organic osmolytes the contribution of soluble sugars was 10%, whereas the proline and glycine betaine contribution was <1% in osmotic adjustment (data not shown). Similar

Table 3. Changes in leaf osmotic adjustment under salinity

Osmotic adjustment (total osmotic adjustment: OA_t , active osmotic adjustment: AOA, passive osmotic adjustment: POA) and percent contribution of sodium (Na^+), potassium (K^+), chloride (Cl^-), soluble sugar to leaf osmotic potential of *Phragmites karka* treated with 100 and 300 mM NaCl for 15 days

Parameters	NaCl	
	100 mM	300 mM
<i>Inorganic</i>		
Na^+	3.9 ± 0.5	3.7 ± 0.5
K^+	22.0 ± 1.4	24.8 ± 0.6
Cl^-	29.8 ± 2.0	29.7 ± 2.0
$\sum (\text{Na}^+ \text{K}^+ \text{Cl}^-)$	55.7 ± 3.4	58.2 ± 3.5
<i>Organic</i>		
Soluble sugar	9.1 ± 1.0	9.0 ± 0.9
Others	25.2 ± 3.6	22.8 ± 6.2
<i>Osmotic adjustment</i>		
OA_t	0.8 ± 0.1	1.0 ± 0.1
AOA	0.8 ± 0.1	0.8 ± 0.0
POA	0.0 ± 0.2	0.2 ± 0.1

Table 4. Pearson correlation among different parameters of the pressure volume curve

Bulk elastic modulus (ϵ), osmotic potential at full turgor (Ψ_{S_0}), osmotic potential at turgor loss point ($\Psi_{W_{TLP}}$) and growth attributes [leaves fresh weight (LFW) and plant fresh weight (PFW)]. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., non-significant

Parameters	Ψ_{S_0}	$\Psi_{W_{TLP}}$	LFW	PFW
ϵ	-0.4n.s.	-0.7*	-0.8**	-0.7*
Ψ_{S_0}	-	0.9***	0.5n.s.	0.5n.s.
$\Psi_{S_{TLP}}$	-	-	0.8*	0.7*
LFW	-	-	-	0.9**

results were also reported in *P. australis* (Gorai *et al.* 2011; Maimaiti *et al.* 2016). However, there is a possibility that polyols (especially mannitol) may contribute to osmotic adjustment in *P. karka* as also reported in *Phragmites australis* (Maimaiti *et al.* 2016) and *Phragmites communis* (Briens and Larher 1982). Although small changes in leaf Ψ_π were found at 300 mM NaCl, there was a significant loss in turgor potential (Ψ_p), which suggests that increasing cell wall rigidity may result in poor plant growth (Martinez *et al.* 2004; Verslues *et al.* 2006).

Some plants show significant changes in diurnal leaf water relations (Naidoo *et al.* 2008) compared with others with little variations (Turner and Long 1975; Wenkert *et al.* 1978; Hinckley *et al.* 1980). These changes could be a result of both higher tissue rigidity and decreases in osmotic potential (Touchette 2006; Rozema and Schat 2013). Plants having less elastic cell walls but with greater fluctuations in diurnal Ψ_w , may be termed as ‘drought avoiders’ with ‘water spending strategy’ whereas those showing little variations in diurnal water relations tend to have a ‘water conserving strategy’ and are known as ‘drought tolerant’ (Salleo 1983; Gullo *et al.* 1986; Gullo and Salleo 1988). A rapid change in diurnal Ψ_w (decrease in mid-day Ψ_w) indicates that *P. karka* was able to quickly pull water from the soil, which suggests a water spending strategy (Salleo 1983). The differences between pre-dawn and mid-day leaf Ψ_w and Ψ_π were even higher in 300 mM NaCl, which indicates poor drought resistance in *P. karka* (Bolaños and Longstreth 1984;

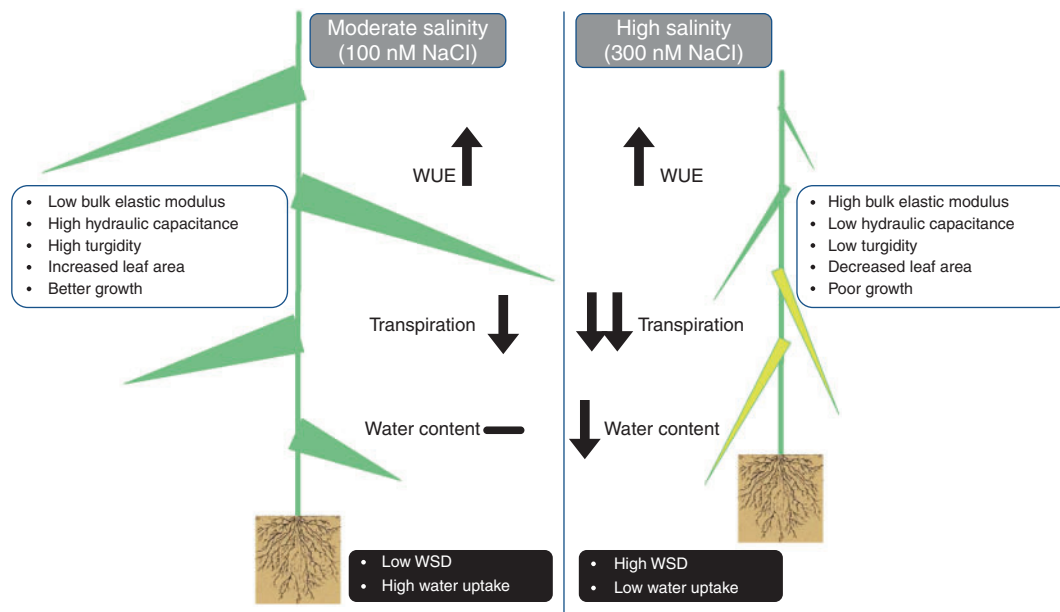


Fig. 7. Model representing the changes in different parameters of *Phragmites karka* after treatment with 100 and 300 mM NaCl. The direction of the arrows shows the change in comparison with the non-saline treatment and the number of arrows indicate a significant difference ($P < 0.05$) between salinity treatments.

Pardossi *et al.* 1998; Touchette 2006). However, to confirm this assumption, further research would be required under drought and flooding conditions coupled with salinity.

In our experiment, the PVC data showed higher ΨP_0 at 100 mM NaCl, which is in agreement with higher solute accumulation (more negative ΨS_0) that contributed to lower ΨW_0 (Touchette *et al.* 2009). Moreover, the lack of a difference in ϵ and WSD at 100 mM NaCl compared with the non-saline control indicates non-disruptive water balance (Touchette 2007). Plants also increased WUE_i to strictly regulate water loss from aerial tissues in order to maintain water balance. A 50% reduction in ΨP_0 in 300 mM NaCl is linked to a 3-fold increase in ϵ of cell wall and lower ΨW_{TLP} (Zheng *et al.* 2010; Suarez 2011), which indicates plant sensitivity to water stress (Bolaños and Longstreth 1984; Pardossi *et al.* 1998; Lenz *et al.* 2006; Touchette 2007; Touchette *et al.* 2009). Differences between ΨW_{TLP} and leaf Ψ_π were lower in 300 mM NaCl compared with 100 mM NaCl, suggesting that plants are unable to pull water from the soil, which is well supported by increases in WSD and reduced leaf growth, transpiration rate and hydraulic conductance (Bartlett *et al.* 2012). *Phragmites karka* was also unable to further decrease leaf Ψ_π at 300 mM compared with 100 mM NaCl (as indicated by unchanged OA_t between 100 and 300 mM NaCl). Most of the moderately salt-tolerant grasses of salt marshes display a similar response (for example, *Andropogon greenwayi*: Hamilton *et al.* 2001; *Sporobolus tremulus*: Moinuddin *et al.* 2014), whereas highly salt-tolerant grasses gradually decrease Ψ_π with increases in salinity (*Urochondra setulosa*: Gulzar and Khan 2008; *Aeluropus lagopoides*: Moinuddin *et al.* 2014). Bartlett *et al.* (2012) also reported that different responses in leaf Ψ_π depend on the type of species and habitat in which plants naturally grow. *Phragmites karka* increased solute accumulation (decrease in

ΨS_0) at 100 mM NaCl (Abideen *et al.* 2014) indicating high dependency on active osmotic adjustment (AOA) (Salpeter *et al.* 2012). However, at 300 mM NaCl plants maintained OA_t by increasing cell wall rigidity thus restricting the entry of both water and solutes, which could result in increased apoplastic water fraction (Touchette *et al.* 2009; Salpeter *et al.* 2012; Flowers *et al.* 2015; Hessini *et al.* 2015). Increasing apoplastic water represents passive osmotic adjustment (POA), which is considered a useful strategy against the negative effects of salinity in general (Navarro *et al.* 2007) and for avoiding oxidative stress in particular (Miller *et al.* 2010). However, POA in plants could be at the cost of cellular dehydration (Flowers and Yeo 1986), which is in accordance with high WSD. Increase in WSD at 300 mM NaCl appear to be linked with ϵ but not with sclerophylly indices like LTD and SUC, as observed in other monocots (Parida and Das 2005; Gorai *et al.* 2010; Rozema and Schat 2013). In addition, increased ϵ is the possible reason for the decline in hydraulic capacitance of leaf tissues under 300 mM NaCl, i.e. the difference between C_{FT} and C_{TLP} in 300 mM ($0.13 \text{ mol m}^{-2} \text{ MPa}^{-1}$) compared with 0 and 100 mM NaCl ($2.5 \text{ mol m}^{-2} \text{ MPa}^{-1}$). A strong negative correlation of ϵ with ΨW_{TLP} , LFW and PFW indicates a greater role of cell wall rigidity in *P. karka* under physiological drought. Our findings are in agreement to the above mentioned hypothesis that 'growth response of *P. karka* under salinity depends on the bulk elastic modulus'.

Conclusion

This study provides a detailed explanation of water relations of *P. karka* under salinity. Plants maintained turgor under moderate salinity (100 mM NaCl) by low tissue rigidity, increased hydraulic capacitance and active osmotic adjustment, which

ultimately facilitated in maintenance of water balance and growth (Fig. 7). However, leaf wilting and poor plant growth at 300 mM NaCl is the consequence of greater diurnal fluctuations in water potential, low transpiration, high water saturation deficit and tissue rigidity (Fig. 7). Water deprivation at 300 mM NaCl indicated poor drought tolerance compared with non-saline control and 100 mM NaCl. In conclusion, it is recommended that *P. karka* could be grown on marginally saline lands particularly for producing biomass for biofuels. This study also provides a new dimension for future research to understand the role of cell wall rigidity on growth, with the help of modern molecular tools, which may be useful to enhance the salt tolerance of giant grasses.

Conflicts of Interest

The authors declare no conflicts of interest.

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