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## The Effect of Sodium Chloride Salinity on the Growth, Water Status and Ion Content of *Phragmites communis* Trin

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**Abstract:** The present study deal with the physiological behavior of *Phragmites communis* under salt stress. The effects of salinity on growth, dry weight partitioning, water status and ion content were studied on seedlings of *P. communis* fed with nutrient solutions containing 0 to 600 mM NaCl. The plants grew best when irrigated with distilled water; biomass production and Relative Growth Rate (RGR) decreased with increasing salinity. Nevertheless, plants were able to produce and allocate dried matter to all their organs even at the highest salt level (600 mM NaCl). The leaves showed the lowest growth activity. Increasing salinity was accompanied by a decrease in seedling water content; aerial parts were more dehydrated than roots. Examination of the K<sup>+</sup>/Na<sup>+</sup> selectivity revealed that salt tolerance of reed plants may be due to its capacity to limit Na<sup>+</sup> transport and to enhance K<sup>+</sup> transport into aerial parts resulting in a high K/Na ratio. Our results suggest an exclusive behavior towards Na<sup>+</sup> as shown by the decreasing Na<sup>+</sup> gradients from leaves to roots. It is concluded that Na<sup>+</sup> exclusion mechanism appeared to be operative and contributes to salt tolerance of *Phragmites*.

**Key words:** Growth, NaCl, K<sup>+</sup>/Na<sup>+</sup> ratio, salt tolerance, *Phragmites communis*

### INTRODUCTION

*Phragmites communis* Trin., known as common reed, has wide ecological and geographical amplitudes in Tunisia and grows under a variety of environmental conditions. This wide distribution may imply a high genetic diversity. Salinity generally suppresses common reed's growth (Lissner and Shierup, 1997; Burdick *et al.*, 2001), but differing levels of salt tolerance among ecotypes have been reported (Waisel, 1972; Batanouny *et al.*, 1991). However, little is known about the intraspecific variability of this species. Although *P. communis* is not a true halophyte, it is salt tolerant and can grow on soils having a salt concentration of up to 850 mM (Matoh *et al.*, 1988). It invades salty marshes both through rhizome growth from the upland regions and through seed germination in sparsely populated patches (Wijte and Gallagher, 1996). Similarly, *P. communis* invades coastal wetlands when salinity levels drop (Hellings and Gallagher 1992; Chambers *et al.*, 1999). Salinity reduces the ability of plants to take up water, causing a reduction in growth along with a suite of metabolic changes (Munns, 2002). Salt tolerance is commonly expressed as the percent of biomass production in saline versus control conditions over a

prolonged period of time. It can also be measured in terms of survival. Mauchamp and Mésleard (2001) found that seedlings growing in 340 mM NaCl solution suffered mortality and were too small to survive in the field.

Salt tolerance is not exclusively correlated with adaptation to Na<sup>+</sup> toxicity *per se* but also reflects adaptations to secondary effects of salinity (Flowers *et al.*, 1977; Greenway and Munns, 1980). K<sup>+</sup> uptake is particularly important due to the chemical similarities between Na<sup>+</sup> and K<sup>+</sup>. The capacity of plants to counteract salinity stress strongly depends on the status of their potassium nutrition (Levignron *et al.*, 1995; Munns, 2002). Therefore, one of the key elements in salinity tolerance is the ability to maintain a high cytosolic K<sup>+</sup>/Na<sup>+</sup> ratio (Yeo, 1998; Maathuis and Amtmann, 1999). The aim of the present study was to investigate the effects of salinity on growth, water content and mineral nutrition of *P. communis*.

### MATERIALS AND METHODS

Seeds of *P. communis* (Trin.) obtained from plants which were originally collected from saline location in Zirkiné, Gabès (Tunisia). One thousand seeds weighed, on average, 100 mg. Seeds were germinated on moist organic soil (Table 1). Seedlings were transplanted when they developed one leaf (after about 2 weeks) into 3 L

Table 1: Chemical-physical characteristics of the soil used

Dry matter	25% of substrate	
Organic matter	20% of substrate	
Water retention capacity	80% of substrate	
Conductivity	0.5 dS m <sup>-1</sup> (mmhocm <sup>-1</sup> )	
pH	6 to 6.5	
	Exchangeable	Soluble
Elements	(mmol/100g of substrate)	
Calcium	5.83	0.83
Potassium	0.15	0.28
Sodium	0.07	0.06
chloride	-	0.16

pots (20 cm diameter) filled with the same substrate. The experiments were carried out in a greenhouse where day/night temperature was set to 30/20°C and the photoperiod was set to 14 h. The seedlings were irrigated with distilled water during the first nine weeks then with various NaCl solutions (concentration: 0, 100, 200, 300, 400, 500 and 600 mM) for the next five weeks. Each treatment was applied to 14 single replicate plants. All plants were harvested at the age of 14 weeks. Fourteen control plants were harvested before NaCl treatment in order to calculate relative growth rates. Upon harvest, plants were briefly rinsed with distilled water, blotted dry, then divided into leaves, stems and roots. These fractions were weighed then oven dried at 70°C for 3 days. Fresh Weight (FW) and Dry Weight (DW) of different tissues of each plant were determined after counting the leaf number. Tissue Water Content (WC) was determined. Relative Growth Rate (RGR) was calculated from the dry weight values according to the equation:  $RGR = (\ln DW_2 - \ln DW_1) / (t_2 - t_1)$

Where DW<sub>1</sub> is the dry weight (g) at the time of application of treatments (initial biomass), DW<sub>2</sub> is the final dry weight (g) (final biomass) and (t<sub>2</sub>-t<sub>1</sub>) is the duration of the treatment (35 days).

The Index of Sensitivity (IS) was calculated from the difference in final biomass (g DW) between treated and untreated plants brought back to the final biomass of control plants (Slama, 1986). Dried tissues were finely ground to pass a 40-mesh screen then sub-samples were mineralized with 0.5% nitric acid. Na<sup>+</sup> and K<sup>+</sup> concentrations were measured by flame emission spectrophotometry after nitric acid extraction of the finely grounded dry matter.

The experimental set-up was a completely randomized Design with seven treatments and 14 single replicate seedlings. Data were analyzed using ANOVA in SPSS 11.5 software. Means were separated by Tukey-test at p = 0.05 significance level.

## RESULTS

Shoot elongation was largest on plants irrigated with distilled water (Fig. 1A). Adding NaCl to the irrigation solution significantly reduced shoot growth (p<0.001). For

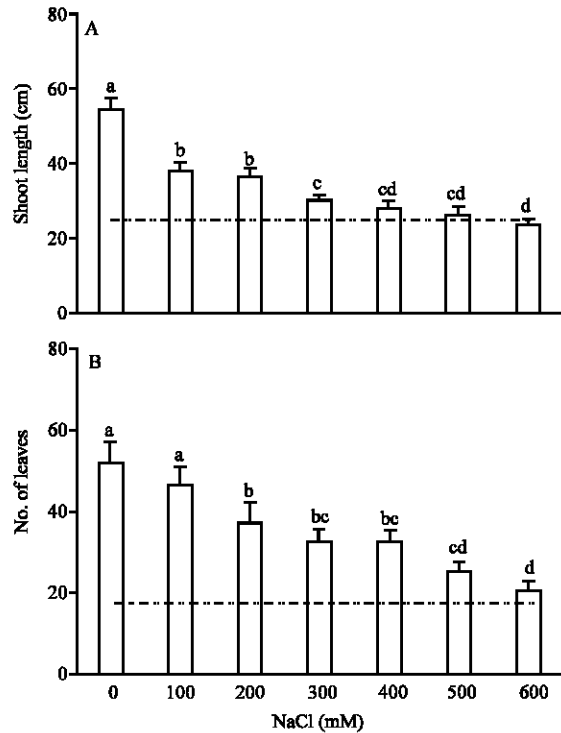


Fig. 1: Shoot length (A) and mean leaf number (B) of 9-week old *Phragmites communis* plants grown for 5 weeks on different NaCl concentrations (0 to 600 mM). The dashed lines correspond to the initial shoot length and leaf number before NaCl treatments. Significant differences between treatments are indicated with different letters (p<0.05, n = 14)

instance, 100 and 600 mM NaCl, reduced shoot length by 30 and 58%, respectively, compared to control plants. Increasing concentrations of NaCl in the irrigation solution significantly reduced the number of green leaves per plant after 5 weeks of treatment (Fig. 1B, p<0.001). Leaf number per plant increased for the controls as new leaves emerged but decreased over time for salinized plants as leaves began to senesce and leaf emergence was delayed. Marginal yellowing of the leaves became apparent at concentration above 300 mM.

Five weeks of irrigation with saline solutions reduced the biomass of all organs (Fig. 2, p<0.001); however, stems and roots were more sensitive than leaves which were not affected up to 200 mM NaCl concentrations. With the 100 mM NaCl solution, stems and roots DW were reduced by 35 and 50%, respectively. Nevertheless, plants were able to produce and allocate dry matter to all organs even at the highest NaCl concentration. The root/shoot dry weight ratio decreased with 100 mM NaCl then increased with the higher concentrations (Fig. 3).

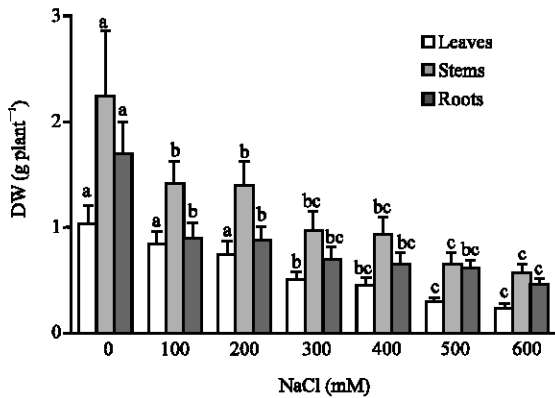


Fig. 2: Effect of salinity on dry weight of roots, stems and leaves of *P. communis* determined on 9-week old plants grown for 5 weeks on various NaCl concentrations. Significant differences between treatments are indicated with different letters ( $p < 0.05$ ,  $n = 14$ )

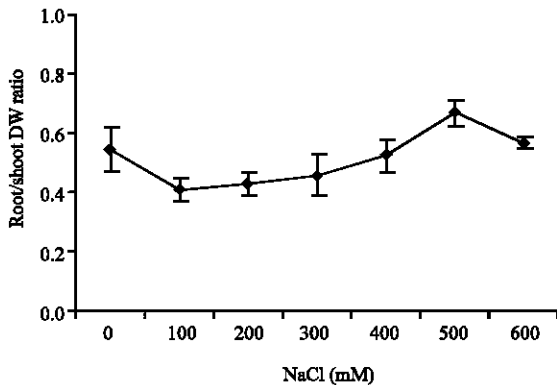


Fig. 3: Effect of salinity on the Root/Shoot dry weight ratio of *P. communis* plants. Means of 14 plants and 95% confidence limits are shown

To better appreciate the effect of salinity on biosynthetic activity of *P. communis* plants, growth was expressed as DW production per unit of time and unit of biomass or relative growth rate (Fig. 4). The RGR decreased with increasing salinity for all three tissues ( $p < 0.001$ ). At all levels of salinity, leaves had the lowest growth activity. The RGR for whole plants averaged 0.03 and 0.01  $\text{g g}^{-1}$  per day at 0 and 600 mM, respectively (data not shown).

As shown in Fig. 5, the presence of NaCl in the medium led to a reduction of leaf and stem water contents ( $p < 0.001$ ) but not that of roots ( $p > 0.05$ ). Root water content was higher than that of stems and leaves. There was a positive correlation between shoot water content and shoot dry weight ( $R^2 = 0.96$ ) (data not shown).

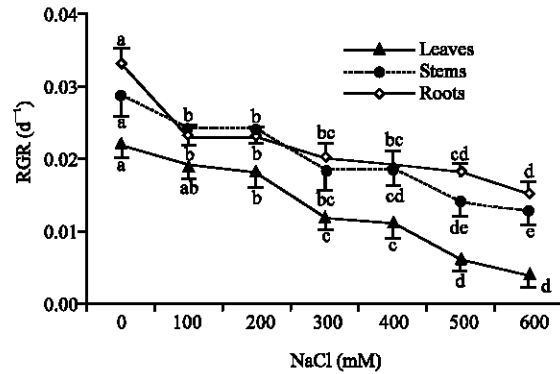


Fig. 4: Relative Growth Rate (RGR), ( $\text{g g}^{-1} \text{DW day}^{-1}$ ) of leaves, stems and roots of *P. communis* plants grown for 5 weeks on various NaCl concentrations. Significant differences between treatments are indicated with different letters ( $p < 0.05$ ,  $n = 14$ )

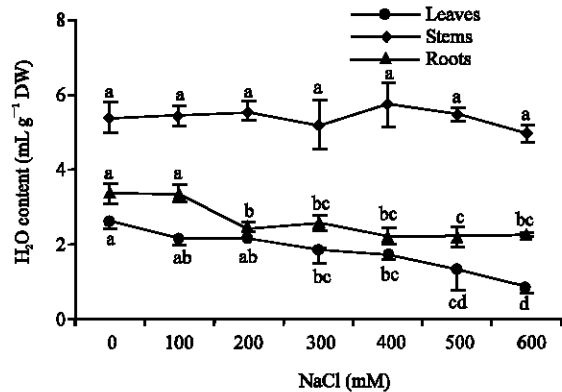


Fig. 5: Water content ( $\text{mL H}_2\text{O g}^{-1} \text{DW}$ ) of *P. communis* plants during acclimatization to the higher salt concentration. Significant differences between the treatments were shown at  $p < 0.05$  with different letters ( $n = 14$ )

To appreciate the sensitivity of *P. communis* to the salinity, the index of sensitivity was determined for the three plant parts (Fig. 6). For NaCl concentrations up to 400 mM, leaves were less sensitive to salinity than stems and roots. For higher concentrations, all plant parts were equally sensitive ( $p < 0.001$ ).

Sodium content of all plant parts increased when NaCl was added to the irrigation solution (Fig. 7,  $p < 0.001$ ), with roots accumulating considerably more than did leaves and stems. With 600 mM NaCl,  $\text{Na}^+$  content of roots was  $1600 \mu\text{mol g}^{-1} \text{DW}$ , whereas that of stems and leaves were 450 and  $280 \mu\text{mol g}^{-1} \text{DW}$ , respectively. Potassium content of leaves and stems increased slightly

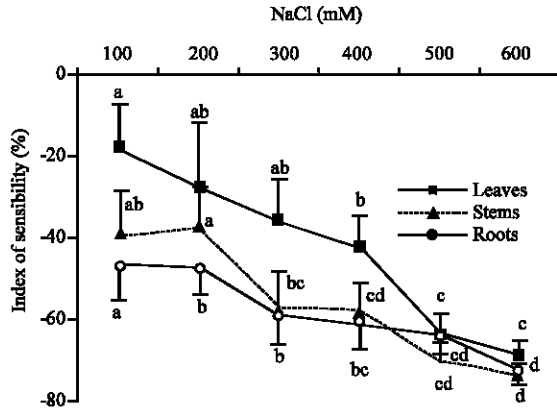


Fig. 6: The index of sensitivity of different organs of *P. communis* plants growing at various NaCl concentrations (0 to 600 mM). Means of 14 plants and 95% confidence limits are shown

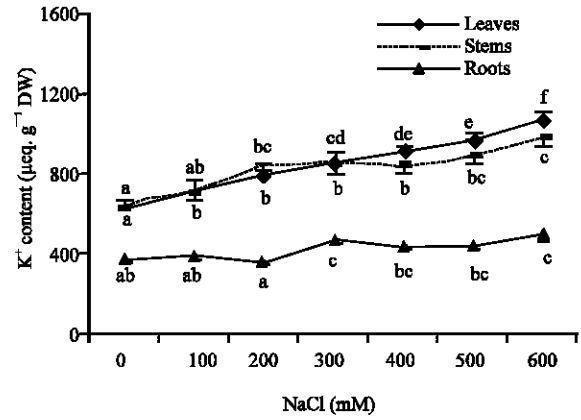


Fig. 8: Potassium contents of leaves, stems and roots of 9-week old *P. communis* plants grown for 5 weeks on various NaCl concentrations. Means of 14 plants and 95% confidence limits are shown

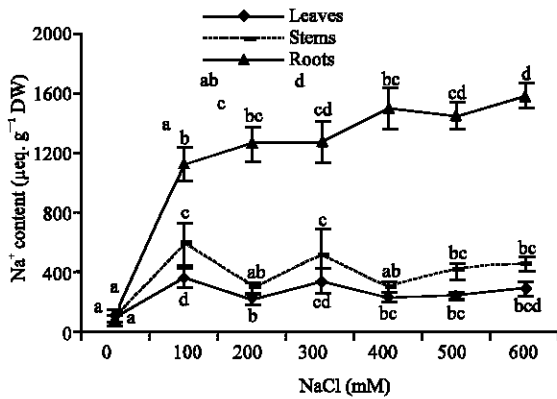


Fig. 7: Sodium contents of leaves, stems and roots of 9-week old *P. communis* plants grown for 5 weeks on various NaCl concentrations. Means of 14 plants and 95% confidence limits are shown

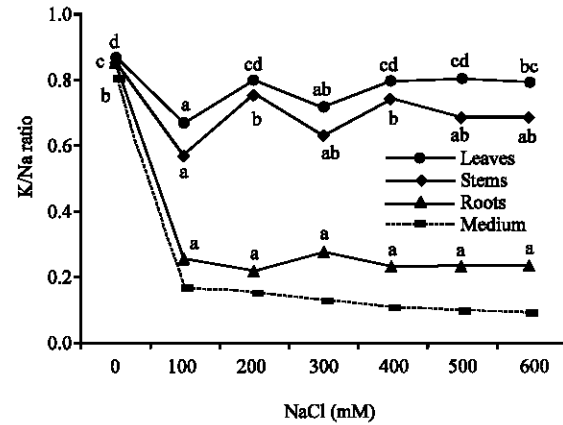


Fig. 9: Effect of salinity on the  $K^+/Na^+$  ratio in leaves, stems and roots of *P. communis* plants, after 5 weeks of culture in the presence of different NaCl concentrations (0 to 600 mM) in the medium

with increasing NaCl concentrations in the growth solution (Fig. 8,  $p < 0.001$ ). Root  $K^+$  remained almost unchanged.  $Na^+$  was more abundant than  $K^+$  but less abundant in the leaves. The  $K/Na$  ratio was higher in the aerial parts ( $p < 0.001$ ) than in the roots ( $p < 0.001$ ) (Fig. 9), indicating the ability of the aerial parts to discriminate against  $Na^+$ .

To better understand this mechanism of exclusion, we examined the relationship between  $Na^+$  uptake and biomass accumulation in the whole plant and in its aerial parts (data not shown). In terms of the effect of salinity on accumulation of sodium, it is clear that significant differences existed among the aerial parts and whole plant. Our experiments showed that sodium enrichment of aerial

parts makes him self in the same way as the whole plant. However,  $Na^+$  uptake by the aerial parts decreased with increasing salinity. We recorded only the half of sodium quantities in aerial parts compared with the whole plant. This behavior could be considered that roots function like a regulator in  $Na^+$  leaves enrichment.

## DISCUSSION

Growth of *P. communis* seedlings was reduced by increasing NaCl concentrations in the irrigation solution (Fig. 2). This was in agreement with previous reports (Match *et al.*, 1988; Hellings and Gallagher, 1992; Lissner

and Shierup, 1997; Mauchamp and Mésleard, 2001). Leaf number, shoot length and dry mass of leaves, stems and especially roots were all reduced (Fig. 1 and 2). The differences in salt sensitivity among different plants parts translated into different root/shoot dry weight ratios. Our results suggest that roots are sensitive to even relatively low salt levels (100 mM NaCl) whereas shoots become more susceptible at higher concentrations (500 and 600 mM) (Fig. 3). Root sensitivity to NaCl resulted in a lower RGR with increasing salt concentrations (Fig. 4). Match *et al.* (1988) showed that RGR decreased by 83% at 250 mM NaCl in the growing medium. Daniels (1991) showed that *P. communis* from different origins had different growth rates.

*P. communis* appears, thus, able to maintain a high selectivity in favour of K<sup>+</sup> when salinity increases in the medium; this explains its halophytic behavior (Fig. 9). The capacity of plants to main a high cytosolic K/Na ratio is one of the key determinants of plant salt tolerance (Maathuis and Amtmann, 1999). Although it's salt tolerant, *P. communis* grows less than its potential in the presence of salt (Lissner and Shierup, 1997; Chambers *et al.*, 1999, Mauchamp and Mésleard, 2001). Under natural conditions, it grows best in fresh water; however, it can survive under saline conditions approaching those of sea water thus its halophytic behavior (Match *et al.*, 1988; Mauchamp and Mésleard, 2001).

There was a strong positive correlation between shoot water content and its DW. The values of water contents we found in our study were lower than those reported by Match *et al.* (1988). The difference may be due to the growing conditions. We used a solid substrate whereas they used a hydroponic system. The sensitivity of roots even at low NaCl concentrations (100 mM) suggests that they are the seat of sensitivity to salinity for this species (Fig. 6). Therefore, the reduction of water content of shoots may be due to an inadequate supply of water from roots (Fig. 5).

The depressive effect of salinity on plant growth is commonly due to an unbalance of water and/or nutritional relations (Flowers *et al.*, 1977). In our study, there was no correlation between the RGR and K/Na selectivity in shoots (data not shown). Therefore, the limitation on growth may be osmotic in nature. Levitt (1972) considers that the osmotic effect is the major factor limiting growth. The inaptitude of plants to hydrate their tissues appropriately on saline soils causes a physiological drought. The most salt tolerant plants accumulate Na<sup>+</sup> in their leaves whereas others don't; the first group are called inclusions the second are called excluders (Slama, 1986). *P. communis* accumulates sodium in its

roots away from its aerial parts; it behaves like an excluder thus not a truly salt tolerant plant (Fig. 7). Nevertheless, it is more tolerant than glycophytes. Similar results were reported by Match *et al.* (1988) and Matsushita and Match (1991). It is possible that roots regulate Na<sup>+</sup> uptake. Match *et al.* (1988) reported that the most conspicuous feature of salt-grown reed plants was their ability to restrict Na<sup>+</sup> intrusion into the shoots.

In most halophytes, Na<sup>+</sup> accumulates in leaf vacuoles, thus lowering their water potential, increasing the driving force for water uptake into the vacuoles and thus increasing cell turgor (Flowers *et al.*, 1991). In our study, leaf water content fell as sodium concentration rose although not as much as in roots (Fig. 10). Two mechanisms may be in play here. First, it is possible that a proportion of Na<sup>+</sup> ions which accumulated within the leaves remained in the cell walls, bringing about an efflux of water from the protoplast to the apoplast and the subsequent loss of this water by transpiration (Flowers *et al.*, 1991). Second, Na<sup>+</sup> ions may be sequestered in root cell vacuoles hence the stable water content of these tissues. This corroborates previous a report by Matsushita and Match (1991) suggesting that the lower Na<sup>+</sup> transport from roots to shoots can limit excessive Na<sup>+</sup> accumulation in shoots. They concluded that common reed has an exclusive behavior towards Na<sup>+</sup>.

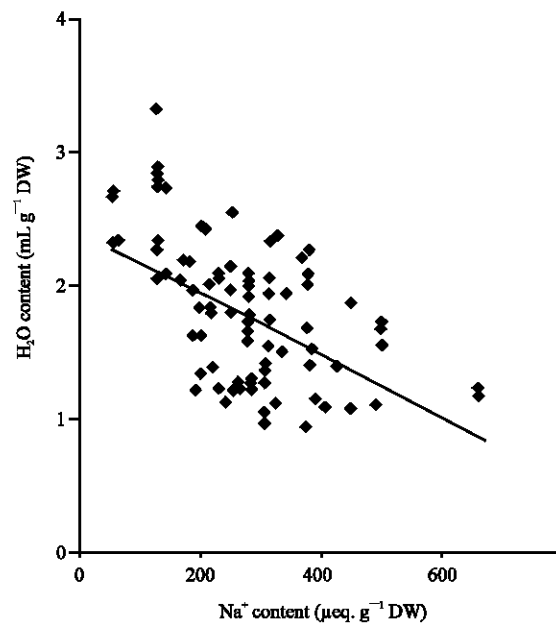


Fig. 10: The relationship between sodium and water contents of leaves of 9-week old *P. communis* plants grown for 5 weeks on various NaCl concentrations (0 to 600 mM). Data from all treatments were pooled

The ability of this species to maintain a substantial growth rate under saline conditions is directly related to an efficient K/Na selectivity. Moreover, *P. communis* tolerance was due to its capacity to limit Na<sup>+</sup> transport and to enhance K<sup>+</sup> supply to the shoots. The limiting effect of salinity on growth of this species appears to be osmotic in nature. Yeo (1998) suggested that dry matter production is proportional to leaf K<sup>+</sup> nutrition. Leaf K<sup>+</sup> content and K<sup>+</sup>/Na<sup>+</sup> selectivity in the aerial parts can be considered good indicators of the performance of plants in saline conditions (Greenway and Munns, 1980; Maathuis and Amtmann, 1999) and therefore they can be used as precocious criteria to select cultivars for NaCl tolerance.

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