Pak. J. Bot., 40(2): 799-805, 2008.

GROWTH AND IONIC COMPOSITION OF SALT-STRESSED *EUCALYPTUS CAMALDULENSIS* AND *EUCALYPTUS TERETICORNIS*

MUHAMMAD NASIM[1*], RIAZ H. QURESHI[2], TARIQ AZIZ[1], M. SAQIB[2], SHAFQAT NAWAZ[1], SHAHBAZ TALIB SAHI[1] AND SHAHID PERVAIZ[2]

[1]*University of Agriculture, Sub-Campus Depalpur Depalpur, Okara*
[2]*Saline Agriculture Research Centre, University of Agriculture Faisalabad-38040, Pakistan*
[*]*Corresponding author E-mail: mnasimshahid@yahoo.com*

Abstract

The effect of NaCl salinity on growth, biomass and ionic composition of *Eucalyptus camaldulensis* and *E. tereticornis* was studied in a nutrient solution experiment. Species were grown with control (no NaCl) and 150 mol m^{-3} NaCl salinity in Hoagland nutrient solution. Salinity significantly suppressed plant height, shoot and root fresh biomass in both the species. However, degree of reduction in these growth parameters was significantly different among both species. Relative fresh weight of *E. camaldulensis* under salt stress was significantly more than of *E. tereticornis*. Salinity stress caused significant effect on Na, K and Cl uptake and its distribution within mature and young leaves. Sodium concentration was about 4.5 folds higher in plants grown with salinity than those grown without salinity. Potassium concentration was significantly decreased in both eucalyptus species under salinity stress. However, salt induced reduction in K concentration was significantly lower in *E. camaldulensis*. Based on the relative fresh weight, K:Na ratio, ion distribution in young and mature leaves, *E. camaldulensis* exhibited more tolerance against salinity than *E. tereticornis*.

Introduction

Salinity is a major abiotic stress to plant growth which adversely affects agricultural productivity and environmental health. Salt affected soils are common feature of the irrigated agriculture over the world particularly in arid and semi-arid regions (Ashraf, 2004). Excess of soluble salts in root zone severely affect growth of higher plant species mainly glycophytes (Ashraf & Harris, 2004; Raza *et al.*, 2006). Salt stress causes nutrient imbalance in plants by influencing uptake, transport and utilization of different nutrients (Grattan & Grieve, 1999; Zhu, 2003) which may result in an excessive accumulation of Na^+ and Cl^- in tissues (Ali *et al.*, 2006a). Concentration of K in plants decline as Na salinity in the root media is increased (Saqib *et al.*, 2004; Ali *et al.*, 2006). Hence, maintenance of adequate K contents in leaves is essential for plant survival in saline soils.

Species and varieties differ greatly in their response to salinity in root medium (Saqib *et al.*, 2005; Ali *et al.*, 2006b; Tahir *et al.*, 2006). Difference among species and varieties for salinity tolerance may reside in their differences in salinity tolerance mechanisms such as Na/K selectivity, Na exclusion, compartmentation inside cell, accumulation of osmolytes, protection of cell integrity and ion homeostasis etc. (Marschner, 1995; Akram *et al.*, 2006; Ali *et al.*, 2006b; Tahir *et al.*, 2006).

Exploitation of these useful genetic variations in salinity tolerance of crop plants/trees is an economical approach for revegetation of salt-affected lands. Plant characteristics such as salt and waterlogging tolerance, adaptation, uses, propagation and management, and productivity are important factors to be considered while selecting tree species for the rehabilitation of salt-affected lands. Eucalyptus is an important tree species tolerant to salinity, thus has great potential for rehabilitation of salt-affected wastelands (Rawat & Banerjee, 1998). In an adaptation trial near Faisalabad, the *Eucalyptus* spp., performed better than 11 other tree species studied over seven and half years (Qureshi *et al.*, 1993). Screening of 13 *Eucalyptus* species showed differences in salt tolerance in a preliminary solution culture experiment. Species were categorized into four groups on the basis of their growth performance viz., tolerant, moderately tolerant, moderately sensitive and sensitive species. Two species, one from tolerant (*Eucalyptus camldulensis* (Silverton)) and one from moderately tolerant *(Eucalyptus tereticornis)* group were selected for this experiment to reconfirm their growth performance under salinity and to evaluate the effect of NaCl salinity on their ionic composition and ionic distribution within different aged leaves. The relative tolerance against salinity of these two species was determined to assess the suitability of *Eucalyptus* species for afforestation and revegatation of wastelands.

Materials and Methods

The experiment was conducted to study growth response and ionic composition of two *Eucalyptus* species (*E. camaldulensis*) and *E. tereticornis*) as affected by salinity stress in a rain-protected wire house at Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad, Pakistan (73.4° longitude and 31.5° latitude). The average day and night temperatures during the study were 30 and 18°C, respectively while the average maximum relative humidity was 65%. There was 7 h daily sunshine and light intensity varied between 300 and 1300 µmol photon $m^{-2}S^{-1}$ depending upon day and cloud conditions during the growth period.

Plant growth: Seeds of both species were collected from Australian Re-vegetation Corporation Limited, Western Australia. Fifty seeds of each species were sown in polyethylene lined iron trays containing silica gravel and synthetic vermiculite (mixed in 1:1 ratio) moistened with canal water (Electric conductivity = 0.29 dSm^{-1}). One week after germination, half strength Hoagland nutrient solution (Hoagland & Arnon, 1950) was used for seedling establishment. Three months old uniform sized seedlings were transplanted in foam plugged holes on thermopore sheets floating on continuously aerated (with aquarium pumps) half strength Hoagland nutrient solution. Solution was contained in two polyethylene lined iron tubs of 200 L capacity (100 X 100 X 30 cm^3). There were two seedlings per repeat and each species was replicated five times in each treatment following completely randomized design (CRD). Solution pH was monitored and maintained daily at 6 ± 0.5. Seedlings were grown for 15 days under normal conditions. Thereafter 150 mol m^{-3} Sodium chloride (NaCl) was added in one tub in three equal splits (each of 50 mol m^{-3} NaCl after two days) in a week. Other tub was kept as control to compare the growth of salt-affected plants with normal grown plants. The treatment solution was replaced thereafter weekly till harvesting. After five weeks of salt stress, plant height was recorded using simple measuring tape.

Harvesting and plant analysis for ionic composition: Plants were harvested after five weeks of salt stress. Shoot and root fresh weight was recorded after washing the plant samples. Leaves of plants were divided into top leaves (young leaves) and lower fully expanded leaves (mature leaves) to see ionic distribution between plant parts. The separated plant parts were immediately washed with distilled water and blotted dry with tissue papers. The samples were then dried at 70°C in a forced air oven for 48 h. The oven dried plant samples were fine ground in a wiley mill to pass through 1 mm sieve. The ground plant samples (1 g) were digested in tri-acid mixture (nitric acid, sulfuric acid and perchloric acid) on a hot plate to determine potassium (K) and sodium (Na) concentration (Miller, 1998). Potassium and Na were determined on a flame photometer (Jenway PFP-7). For chloride determination, samples were extracted with dil. HNO_3 (Rashid, 1986). Chloride was determined from this extract on chloride analyzer (Corning Chloride Analyzer 926).

Statistical analysis: The salinity and species were arranged in a 2X2 completely randomized design. The data was analyzed statistically using PC based program MStat-C (Michigan State University, 1996). Comparison among different means of salinity levels and species were evaluated by analysis of variance for growth parameters and ionic composition of plants (Steel & Torrie, 1980).

Results and Discussion

Growth parameters: There were significant main and interactive effects of species and salinity on plant height (PH), shoot fresh weight (SFW) and root fresh weight (RFW) of *Eucalyptus* spp., (Fig 1). Variations in growth and salt tolerance between species of woody plants have also been reported (Qureshi *et al.*, 1993; Rawat & Banerjee, 1998).

Salinity significantly ($p<0.01$) decreased Ph in both species (Fig. 1a). Plant height was significantly different among both species grown under salinity stress. However, Ph was not statistically different among species when grown under normal conditions. Relative Ph of *E. camaldulensis* (73%) was significantly more than *E. tereticornis* (50%). Shoot fresh weight of plants grown in control treatment was significantly more (> 2 folds) than those grown with salinity when averaged over both species (Fig. 1b). Difference among both species for SFW at control treatment was non-significant ($p<0.05$). However, both species significantly differed for SFW under salinity treatment. *Eucalyptus camaldulensis* (Silverton) produced higher SFW than *E. tereticornis* at 150 mol m^{-3} NaCl salinity in root medium. Relative fresh weight (percent of control) is considered an important criterion for salinity tolerance (Saqib *et al*., 2004; Tahir *et al., 2006*). Species with higher fresh weight are considered better to grow under saline conditions. Relative shoot fresh weight was significantly more in *E. camaldulensis* than in *E. tereticornis* indicating its higher tolerance against salinity although both have similar plant height (PH) and shoot fresh weight (SFW) in control treatment.

Salinity stress significantly decreased root fresh weight (RFW) of both species. Species differed significantly for RFW at both treatments (Fig. 1c). *Eucalyptus camaldulensis* (Silverton) produced more RFW than *E. tereticornis* at both treatments. Relative root fresh weight (percent of control) in *E. tereticornis* and *E. camaldulensis* was 28 and 62%, respectively. Roots have to take up nutrient and water; hence have to perform more under any type of soil stress (Lynch, 1995). Significant and positive correlation of root fresh weight with SFW ($r = 0.81$, $p<0.05$) clearly indicated the role of roots in combating salinity stress.

Ionic composition: Leave samples were divided into young and mature leaves for their chemical analysis and to compare distribution of different ions within plant tissues. Salinity stress caused significant effect on Na^+, K^+ and Cl^- uptake and their distribution within mature and young leaves in both species (Table 1).

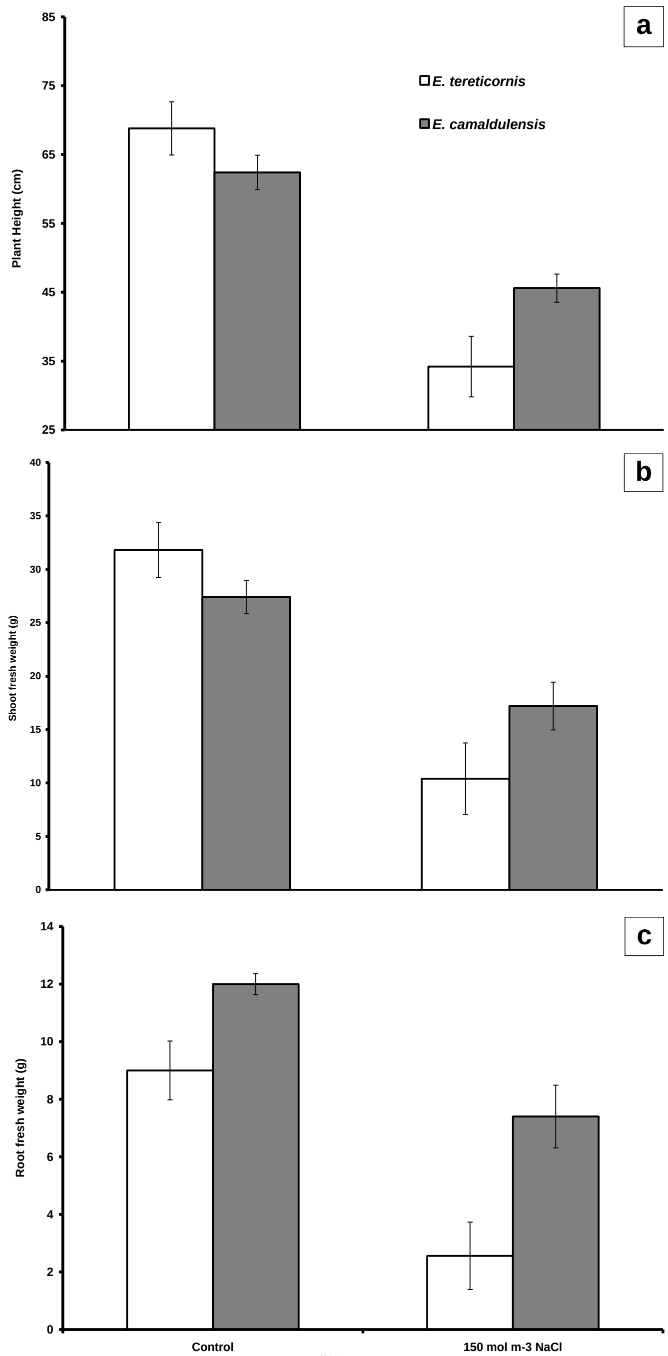

Fig. 1. Plant Height (a), shoot fresh weight (b) and root fresh weight (c) of *Eucalyptus* species grown with and without 150 mol m^{-3} NaCl.

Table 1. Effect of NaCl salinity on sodium, potassium, K/Na ratio and chloride concentration in young and mature leaves of Eucalyptus species.

Species	Na^+ concentration (mmol kg^{-1})			
	Young leaves		Mature leaves	
	Control	150 mol m^{-3}	Control	150 mol m^{-3}
E. tereticornis	155 ± 6^{NS} (0.85)	677 ± 17^{NS} (0.78)	184 ± 10^{NS} -	872 ± 16** -
E. camaldulensis	145 ± 10 (0.85)	666 ± 19 (0.68)	172 ± 4 -	986 ± 20 -
	K^+ concentration (mmol kg^{-1})			
E. tereticornis	324 ± 9** (1.17)	230 ± 7** (1.10)	279 ± 8** -	211 ± 7^{NS} -
E. camaldulensis	409 ± 5 (1.06)	313 ± 4 (1.51)	389 ± 15 -	209 ± 10 -
	K/Na ratio			
E. tereticornis	2.09 ± 0.03*	0.34 ± 0.01**	1.52 ± 0.05**	0.24 ± 0.02^{NS}
E. camaldulensis	2.84 ± 0.13	0.47 ± 0.01	2.26 ± 0.11	0.21 ± 0.03
	Cl^- concentration (mmol kg^{-1})			
E. tereticornis	142 ± 15**	231 ± 14*	243 ± 15*	389 ± 12^{NS}
E. camaldulensis	82 ± 10	274 ± 10	170 ± 10	409 ± 25

Whereas NS = Non-significant, * = Significant at p<0.05 and ** = Significant at (p<0.01). Main affects of salinity and species are highly significant (p<0.01) for Na, K, K:Na and Cl concentration. Signs are used to see the interactive effects of salinity and species on above parameters.

Values are means of five replicates ± standard error. Values in parenthesis are ratios of ion concentrations in young: mature leaves at both treatments). Plants were grown for 8 weeks under saline conditions and leaves were divided into top (young) and fully expanded (mature) leaves.

Sodium concentration in mature leaves was significantly more (>5 folds) in plants grown with salinity compared to control when averaged over both species (Table 1). Salt stress affects uptake, transport and utilization of different nutrients (Marschner, 1995; Grattan & Grieve, 1999), which may results in excessive accumulation of Na^+ and Cl^- in tissue (Saqib *et al*., 2005) and ultimately reduction in crop yield. At control both species had similar Na concentration in mature leaves. However, under salinity stress Na concentration in mature leaves differed significantly between the species. It was significantly more in *E. camaldulensis* than in *E. tereticornis*. Salinity stress significantly increased Na concentration in young leaves; however, species did not differ for Na concentration in young leaves (Table 1).

Transportation (distribution) of Na^+ towards young leaves (metabolically active sites) was determined by calculating ratio between Na^+ concentration in young leaves and mature leaves. At control, both species had almost similar ratio of Na^+ concentration between young and mature leaves, however when plants were subjected to salinity, Na^+ accumulation in young leaves was lower in *E. camaldulensis* than in *E. tereticornis* (Table 1). This preferential salt accumulation in older leaves can be a salt tolerance mechanism that is quite common in many glycophytes (Greenway & Munns, 1980). Tolerant species would avoid salt accumulation in young and metabolically active leaves for their normal functioning as observed in *E. camaldulensis.*

Potassium (K) concentration in mature leaves was significantly (p<0.05) lower in plants grown with salinity irrespective of species (Table 1). Under saline soils, higher levels of external Na^+ interfere with K^+ acquisition limiting plant K uptake (Saqib *et al.*, 2004). Liu *et al*., (2001) reported that high affinity K^+ transporters in *Eucalyptus* may act as low affinity Na^+ transporters under salt stress which may reduce K^+ uptake. Potassium concentration in mature leaves was significantly more in *E. camaldulensis* than *E. tereticornis* when plants were grown under control. However, both species did not differ for K concentration in mature leaves under saline conditions. Potassium concentration was significantly increased in young leaves in *E. camaldulensis*, while ratio between young and mature leaves was decreased in *E. tereticornis*. This clearly indicated that under salt stress, *E. tereticornis* transferred lower amounts of K^+ to the young leaves than as did *E. camaldulensis*.

Addition of NaCl in the nutrient solution significantly reduced K concentration in young leaves of both species. Potassium concentration in young leaves was significantly ($p<0.05$) more in *E. camaldulensis* than in *E. tereticornis* in both control and saline conditions. Relative K concentration in young leaves of plants grown under salinity stress was significantly more in *E. camaldulensis*.

K/Na ratio was significantly ($p<0.01$) reduced in both species in mature as well as in young leaves when NaCl was added in the nutrient solution. A high K/Na ratio in the cytosol is essential for normal cellular functions of plants (Chinnusamy *et al*., 2005) hence is a good indicator of salinity tolerance (Saqib *et al*., 2004). K/Na ratio was significantly ($p<0.05$) more in *E. camaldulensis* in young as well as in mature leaves at control treatment. K/Na ratio was significantly more in *E. camaldulensis* in young as well as in mature leaves in control treatment indicating its efficiency for K^+ selectivity. In saline conditions both species had similar for K/Na ratio in mature leaves, but K/Na differed significantly in young leaves. K/Na was significantly more in *E. camaldulensis* than in *E. tereticornis*.

Chloride concentration in mature leaves of *E. tereticornis* was significantly higher than in *E. camaldulensis* at control, however opposite was case under salinity where Cl^- concentration in mature leaves was more in *E. camaldulensis* than in *E. tereticornis,* although difference was non-significant. Ali *et al*., (2006b) and Tahir *et al*., (2006) also reported significant differences in Cl concentration in brassica and wheat genotypes grown under salt stress. Addition of NaCl in nutrient solution significantly increased Cl^- concentration in young leaves of both species. Chloride concentration in young leaves was about half in *E. camaldulensis* at control than in E. *tereticornis*.

Eucalyptus camldulensis (Silverton) performed better under saline conditions as exhibited by its higher relative shoot and root fresh weight, lower Na translocation towards young leaves, more K concentration in mature as well as young leaves. Hence *E. camaldulensis* can be considered as salinity tolerant species. However, before any recommendations, long term field trials are warranted.

References

Akram, M.S., Q. Ali, H.R. Athar and A.S. Bhatti. 2006. Ion uptake and distribution in *Panicum antidotale* retz. under salt stress. *Pak. J. Bot*., 38(5): 1661-1669.

Ali, Q., H.R. Athar and M. Ashraf. 2006a. Influence of exogenously applied brassinosteroids on the mineral nutrient status of two wheat cultivars grown under salt stress. *Pak. J. Bot*., 38(5):1621-1632.

Ali, Q., H.R. Athar and M. Ashraf. 2006b. Ion transport in four canola cultivars as influenced by salt stress. *Pak. J. Bot*., 38(5):1703-1708.

Ashraf, M. 2004. Some important physiological selection criteria for salt tolerance in plants. *Flora,* 199: 361-376.

Ashraf, M. and P.J.C. Harris. 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci*., 166:3-16.

Chinnusamy, V., A. Jagendorf and J.-K. Zhu. 2005. Understanding and improving salt tolerance in plants. *Crop Sci*., 45: 437-448.

Grattan, S.R. and C.M. Grieve. 1999. Salinity-mineral nutrient relations in horticultural crops. *Scientia Hort.,* 78: 127-157.

Greenway, H. and R. Munns. 1980. Mechanisms of salt tolerance in non-halophytes. *Ann. Rev. Plant Physiol*. 31: 149-190.

Liu, W., D.J. Fairbairn, R.J. Reid and D.P. Schachtman. 2001. Characterization of two *HKT1* homologues from *Eucalyptus camaldulensis* that display intrinsic osmosensing capability. *Plant Physiol.,* 127: 283-294.

Lynch, J. 1995. Root architecture and plant productivity. *Plant Physiol*., 109: 7-13.

Marschner, H. 1995. *Mineral Nutrition of Higher Plants*. Academic Press, London.

Qureshi, R.H., S. Nawaz and T. Mahmood. 1993. Performance of selected tree species under saline sodic field conditions in Pakistan. In: *Towards the rational use of high salinity tolerant plants*. (Ed.): H. Leith and A.A. Masoom 2: 259-269. Kluwer Acad. Publ. Dordrecht, The Netherlands.

Rawat, J.S. and S.P. Banerjee. 1998. The influence of salinity on growth, biomass production and photosynthesis of *Eucalyptus camaldulensis* Dehnh. and *Dalbergia sissoo* Roxb., seedlings. *Plant Soil*, 205: 163-169.

Raza, S.H., H.R. Athar and M. Ashraf. 2006. Influence of exogenously applied clycinebetaine on the photosynthetic capacity of two differently adapted wheat cultivars under salt stress. *Pak. J. Bot.,* 38: 341-351.

Saqib, M., J. Akhtar and R.H. Qureshi. 2004. Pot study on wheat growth in saline and waterlogged compacted soil II. Root growth and leaf ionic relations. *Soil & Till. Res.,* 77: 179-187.

Saqib, M., J. Akhtar and R.H. Qureshi. 2005. Na exclusion and salt resistance of wheat (*Tricticum aestivum*) in saline-waterlogged conditions are improved by the development of adventitious nodal roots and cortical root aerenchyma. *Plant Sci.,* 169: 125-130.

Steel, R.G.D. and J.H. Torrie. 1980**.** *Principles and Procedures of Statistics.* McGraw Hill Book Co., Inc., NY.

Tahir, M.A., Rahmatullah, T. Aziz, M. Ashraf, S. Kanwal and M.A. Maqsood. 2006. Beneficial effects of silicon in wheat (*Triticum aestivum* L.) under salinity stress. *Pak. J. Bot*., 38(5): 1715-1722.

Zhu, J.-K. 2003. Regulation of ion homeostasis under salt stress. *Curr. Opin. Plant Biol*., 6: 441-445.

(Received for publication 10 February 2007)