SCREENING TREES FOR SALINITY TOLERANCE: A CASE-STUDY WITH TEN *EUCALYPTUS* SPECIES

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The present solution culture experiments were conducted in a bid to screen ten eucalyptus species for their growth response to salinity in the rooting environment. Three months old seedlings were grown with and without 150 mol m^{-3} NaCl salinity in a solution culture experiment for eight weeks. Species differed significantly for plant height, shoot and root dry weights at both salinity levels. Increased NaCl salinity significantly suppressed shoot and root biomass with varying degrees. Two species viz. *E. camaldulensis* (Salt sensitive) and *E. occidentalis* (Salt tolerant) were selected on the basis of their relative shoot dry weight. Both of these species were further grown in solution culture experiment to reconfirm growth response and ionic composition as affected by salinity stress. Absolute shoot and root biomass was significantly higher in *E. camaldulensis* as in the first experiment. Growth response of *E. occidentalis* to applied NaCl salinity was not consistent in this experiment as indicated by its relative shoot dry weight. The inconsistent behavior was attributed to higher temperature and relative humidity in second experiment which aggravated the hazardous effects of salinity in this species. Increased NaCl salinity in root environment significantly increased Na^{+} and Cl^{-1} concentration in leaves of both species. However, salinity did not affected K^{+} concentration significantly in *E. occidentalis*. *E. camaldulensis* although exhibited low relative production at high salinity yet performed better in terms of absolute growth at control as well as at 150 mol m^{-3} NaCl salinity. Hence may be recommended to be used for rehabilitation of saline lands.
Keywords: *Eucalyptus*, salinity stress, ionic imbalance, sodium, K:Na ratio

INTRODUCTION

Salinity is a major abiotic factor contributing towards lower growth and yield of higher plants. Salt affected lands are common feature of the irrigated agriculture over the world particularly in arid and semi-arid regions (Szabolcs, 1994). High salt concentration in root zone affects plant growth mainly through osmotic effects on water uptake and specific ion toxicities (Munns, 2002; Munns, 2005). Salinity stress affects nutrient uptake, transport and utilization of different nutrients (Grattan and Grieve, 1999; Zhu, 2003) resulting in an excessive accumulation of sodium (Na^{+}) and chloride (Cl^{-1}) in tissues (Saqib *et al.*, 2005).

A wide array of adaptive mechanisms has been reported in higher plants to cope salinity stress. These adaptive mechanisms include ion exclusion, ion inclusion, ion uptake selectivity, ion homeostasis, compartmentation (Marschner, 1995; Borsani *et al.*, 2003; Flowers, 2004; Saqib *et al.*, 2005). Variations in these adaptive mechanisms are responsible for differences in tolerance or resistance of plants against these stresses. These differences can be exploited to screen and develop more tolerant / resistant plants that can grow on saline soils with economic yields. This selection and or development of salt tolerant plants can sustain growth and yield of higher plants on saline soils. However, threshold salinity level (at which a plant can grow without yield reduction) differ among species and even cultivars of the species (Saqib *et al.*, 2004; Saqib *et al.*, 2005).

A major emphasis is now being given to grow trees on saline lands to prevent desertification. Increased forestation can improve soil health in a number of ways including its impact on soil organic matter, microclimate, reducing evaporation, releasing protons and organic acids in the rhizosphere, decomposition of roots, changing water infiltration and improving soil aeration and porosity. Hence, on highly saline soils plantation of different salinity tolerant trees can better sustain soil health and economic growth than agronomic crops can do (Qureshi *et al.*, 1993).

Trees differ in a number of ways with agronomic crops, hence may respond differently to salinity stress. A lot of work has been conducted on salinity tolerance of agronomic crop around the globe including Pakistan (Aslam, 1993, Borsani *et al.*, 2003; Saqib *et al.*, 2004; Saqib 2005). However, very little is reported on genetic variations for salinity tolerance among tree species in the country. Plant characters such as salt tolerance, adaptation, uses, propagation and management are important factors to be considered while selecting tree species for the rehabilitation of salt affected lands. The present paper reports differences among 10 eucalyptus species for their tolerance against salinity stress on basis of their growth performance in a solution culture experiment. Two species contrasting in salinity tolerance were selected to study growth and ionic composition of these selected species under salinity stress.

MATERIALS AND METHODS

Both experiments were conducted in a rain-protected wire house at Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad (PAKISTAN). The site is situated at 73.4° longitude and 31.5° latitude.

Experiment I

The experiment was conducted to study differential growth response of *eucalyptus* species grown under normal and saline conditions in root zone. The average day and night temperatures during the study were 21.6 and 6.9 °C, respectively while the average maximum relative humidity was 80 % during the growth period. There was 7 h daily sunshine during the growth period. Ten species (Table 1) of Australian and local origin were collected from Australian Re-vegetation Corporation Limited, Western Australia and Saline Agriculture Research Centre, University of Agriculture, Faisalabad. Fifty seeds of each species were sown in polyethylene lined iron trays containing silica gravel and synthetic vermiculite (mixed in 1:1 ratio). The canal water was used to moisten the seeds for proper germination. Electric conductivity of canal water used was 0.29 dS m^{-1}. One week after germination, half strength Hoagland nutrient solution (Hoagland and 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 hole and each species was replicated five times in each treatment following completely randomized design (CRD). Solution reaction (pH) was monitored and maintained daily at 6±0.5. Seedlings were grown for 15 days in similar solutions and thereafter 150 mol m^{-3} sodium chloride (NaCl) was added in one tub in three equal splits in a week (each split after two days). Other tub was kept as control to compare the growth of salt stressed plants with normal grown plants. The treatment solution was replaced thereafter weekly till harvesting. After twelve weeks of transplanting, plant height was recorded using simple measuring tape. Plants were harvested and their shoot and roots were separated. 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 driven oven for 48 h and their oven dried weights were recorded.

Experiment II

Two species *E. camaldulensis* (local) and *E. occidentalis* were selected from experiment I on the basis of contrasting differences in their growth pattern and response to salt stress to study their salt tolerance mechanisms and ionic composition. The experiment was conducted in the same wire house. The average day and night temperatures during the study were 30.6 and 18.3 °C, respectively while the average maximum relative humidity was 63.6 %. There was 7 h daily sunshine during the growth period. These two species were grown under same salinity treatments as in study-I for six weeks. After harvest, plant height, shoot and root fresh weight was recorded after washing the plant samples. The plants were immediately washed with distilled water and blotted dry with tissue papers. The samples were then dried at 70°C in a forced air driven oven for 48 h and their oven dried weights were recorded.

The plant samples (1 g) were digested in tri-acid mixture (nitric acid, sulfuric acid and perchloric acid, in a ratio of 2:2:1) (Miller, 1998). Potassium (K^+) and sodium (Na^+) were determined on a flame photometer (Jenway PFP-7). Plant samples were extracted with dil. HNO_3 while boiling at water bath and then with hot water for three times for chloride determination (Rashid, 1986). Chloride (Cl^{-1}) was determined from this extract on chloride analyzer (Corning Chloride Analyzer 926).

Statistical Analysis

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 and Torrie, 1980).

RESULTS

Experiment-I

There were significant main and interactive effects of species and salinity on plant height, shoot dry matter, root dry matter and root:shooot ratio of plants (Table 1 & 2). Plant height was significantly different among species. Plant height was maximum in *E. camaldulensis* (Local) and *E. camaldulensis* (Silverton) when grown under normal conditions. Salinity significantly ($p<0.01$) decreased plant height in all of species except *E. occidentalis* which showed about similar growth at both treatments. Shoot dry weight of plants grown in control treatment was significantly more (> 2 folds) than those grown with salinity (Table 1) when averaged over all species. However, species differed significantly for shoot dry matter grown either at control or salinity treatment. *E. camaldulensis* (Local), *E. camaldulensis* (Silverton), *E. rudis* and *E.*

Table 1. Plant height and shoot growth of two eucalyptus species grown with control and 150 mol m^{-3} NaCl (Exp. 1). Values are means of five replicates ± standard error. Plants were grown for 8 weeks under saline conditions.

Species	Plant Height**		Shoot dry weight**	
	Control	150 mol m^{-3}	Control	150 mol m^{-3}
E. occidentalis	55 ± 4	54 ± 3	3.51 ± 0.09	3.35 ± 0.08
E. robusta	63 ± 5	28 ± 2	4.60 ± 0.60	1.76 ± 0.30
E. largiflorens	51 ± 4	33 ± 3	1.55 ± 0.27	1.47 ± 0.08
E. melliodora	31 ± 5	21 ± 2	1.01 ± 0.13	0.69 ± 0.12
E. rudis	59 ± 10	35 ± 3	11.47 ± 0.63	3.87 ± 0.43
E. tereticornis	69 ± 5	35 ± 2	10.26 ± 0.66	2.46 ± 0.08
E. campaspe	13 ± 1	9.0 ± 1	0.14 ± 0.02	0.08 ± 0.01
E. camaldulensis (Silverton)	82 ± 6	38 ± 2	14.20 ± 3.67	4.45 ± 0.14
E. camaldulensis (Lake)	65 ± 7	31 ± 3	6.16 ± 0.80	3.17 ± 0.22
E. camaldulensis (Local)	85 ± 4	50 ± 4	14.79 ± 0.16	6.48 ± 1.22

Whereas ** = significant at $p<0.01$. Main effects of salinity and species were significant ($p<0.01$) for plant height, shoot dry matter and root dry matter. Signs are used for interactive effects of salinity and species on the above parameters.

Table 2. Root growth and root:shoot ratio of two eucalyptus species grown with control and 150 mol m^{-3} NaCl (Exp. 1). Values are means of five replicates ± standard error. Plants were grown for 8 weeks under saline conditions.

Species	Root dry weight*		Root:shoot ratio NS	
	Control	150 mol m^{-3}	Control	150 mol m^{-3}
E. occidentalis	0.72 ± 0.10	0.69 ± 0.10	0.20 ± 0.03	0.21 ± 0.03
E. robusta	1.16 ± 0.18	0.35 ± 0.09	0.26 ± 0.03	0.19 ± 0.02
E. largiflorens	0.34 ± 0.03	0.36 ± 0.06	0.26 ± 0.06	0.25 ± 0.06
E. melliodora	0.26 ± 0.03	0.26 ± 0.01	0.26 ± 0.04	0.46 ± 0.13
E. rudis	1.00 ± 0.39	0.74 ± 0.06	0.09 ± 0.04	0.20 ± 0.02
E. tereticornis	1.35 ± 0.11	0.72 ± 0.06	0.13 ± 0.02	0.29 ± 0.02
E. campaspe	0.04 ± 0.01	0.04 ± 0.01	0.30 ± 0.10	0.40 ± 0.10
E. camaldulensis (Silverton)	2.51 ± 0.48	2.51 ± 0.48	0.19 ± 0.02	0.12 ± 0.03

E. camaldulensis (Local)	[illegible]	[illegible]	[illegible]	[illegible]

tereticornis produced higher SDM than all other species at control treatment. All of these species were also efficient in producing SDM at saline conditions. Lowest SDM was exhibited by *E. campaspe* and *E. melliodora* at both treatments. Similarly species differed significantly for root dry matter (RDM) at both treatments. Maximum RDM was produced by *E. camaldulensis* (Silverton), *E. camaldulensis* (Local), *E. rudis* and *E. tereticornis* at control treatment. Under salinity, *E. occidentalis* also produced higher biomass in addition to these species. Root dry matter exhibited by *E. campaspe* and *E. melliodora* was lowest and also it was not affected by salinity treatment. Root shoot ratio was significantly improved under salinity treatment in all the species except for *E. occidentalis*, *E. robusta*, *E. largiflorens* and *E. camldulensis* (Silverton). Two species viz. *E. occidentalis* and *E. camaldulensis* (Local) with contrasting growth response behavior were selected to study ionic composition as influenced by salinity stress. On basis of relative reduction in biomass at salinity treatment, *E. occidnetalis* was salt tolerant while *E. camaldulensis* was salt sensitive.

Experiment II

The experiment was conducted to study ionic composition of selected eucalyptus species as affected by salinity in the root medium. Salinity stress in the root environment significantly suppressed plant height (PH), shoot dry matter (SDM) and root dry matter (RDM) of both eucalyptus species (Table 3). As expected, *E. camaldulensis* (local) performed better than *E. occidentalis* in terms of PH, SDM and RDM at control as well as at saline conditions. Reduction in SDM and RDM was more pronounced in *E. camaldulensis* (Local) than in *E. occidentalis* Plant height was reduced two folds in both species under salinity stress relative to control. Reduction in SDM was about 2 folds in *E. occidentalis* and 5 folds in *E. camaldulensis* (Local) under salinity stress.

Species differed significantly for Na^+ concentration in leaves at both salinity levels. Sodium concentration was higher in *E. occidentalis* at both salinity treatments (Fig. 1). Higher sodium chloride (NaCl) concentration in the root medium significantly increased Na^+ concentration in leaves of both eucalyptus species. However increase in Na^+ concentration significantly more in *E. occidentalis*.

Potassium concentration (K^+) in leaves was significantly different in both species. Contrary to Na^+, it was significantly more in *E. camaldulensis* at both salinity levels (Fig. 2). Salinity stress significantly decreased K^+ concentration in leaves of *E. camaldulensis* only. There was non-significant effect of salinity on K^+ concentration in leaves of *E. occidentalis*.

Chloride concentration in leaves of both species differed significantly at both salinity treatments. Species varied quite differently at both treatments, as Cl^- concentration was more in *E. camaldulensis* at control treatment but reverse was case when plants were subjected to salinity stress (Fig. 3).

Sodium:potassium ratio was quite high in *E. occidentalis* than in *E. camaldulensis* (Fig. 4). Salinity stress significantly increased Na:K ratio in both species, however increase was more pronounced in *E. occidentalis*.

DISCUSSION

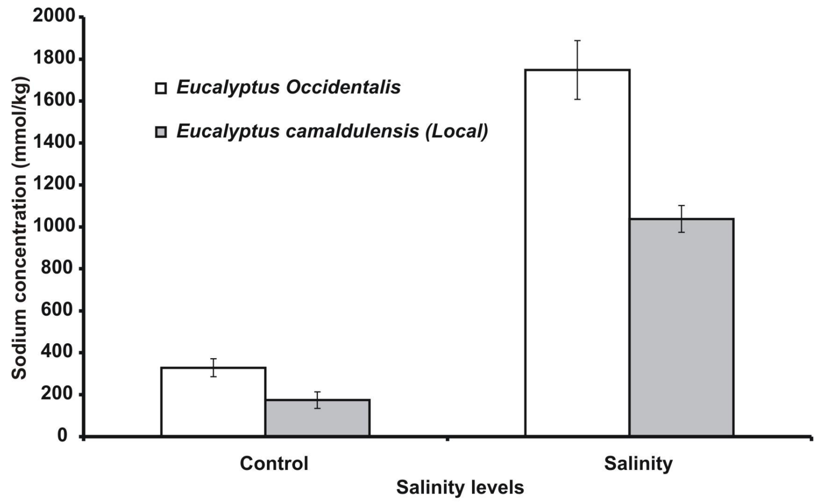

Fig. 1. Sodium concentration in leaves of Eucalyptus speices grown with and without NaCl salinity in root medium

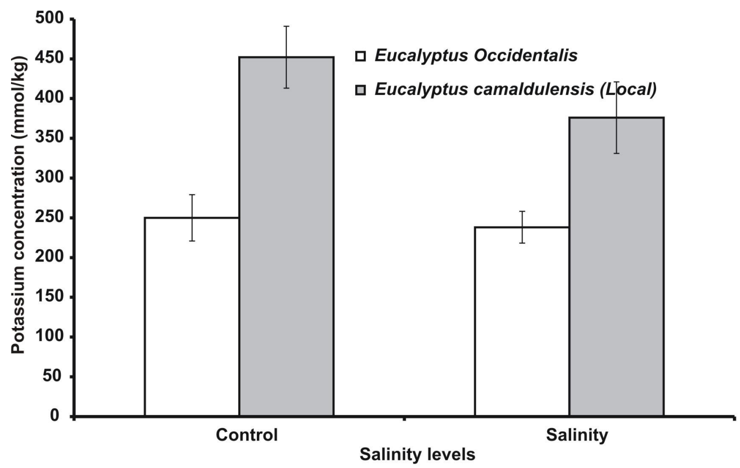

Fig. 2. Potassium concentration in leaves of Eucalyptus speices grown with and without NaCl salinity in root medium

2005). However, very little is reported on salinity tolerance of trees in Pakistan. The present experiments were conducted to evaluate the differences in growth and salinity tolerance of

Eucalyptus spp. from different origins and to identify specific mechanisms for their salinity tolerance. In experiment-I, 10 species were grown under normal and saline conditions in nutrient solution. Species differed significantly for all of the physical parameters when grown either with control and saline conditions. More than seven folds variations were observed among species for their shoot dry matter. Species like *E. camaldulensis* (Local) and *E. camaldulensis* (Silverton) were most efficient in SDM production while *E. campaspe* and *E. melliodora* produced minimum SDM. Salinity significantly affected plant height, shoot and root dry matter and root:shoot ratio of plants. Many of earlier scientists also reported significant reduction in biomass under salt stress in *eucalyptus* (Qureshi *et al.*, 1993; Marcar *et al.*, 1995). However, reduction in SDM due to salinity greatly differed among species as calculated by their relative performance at salinity treatment. Species *E. occidentalis* and *E. largiflorens* were most salt tolerant species in terms of relative growth performance.

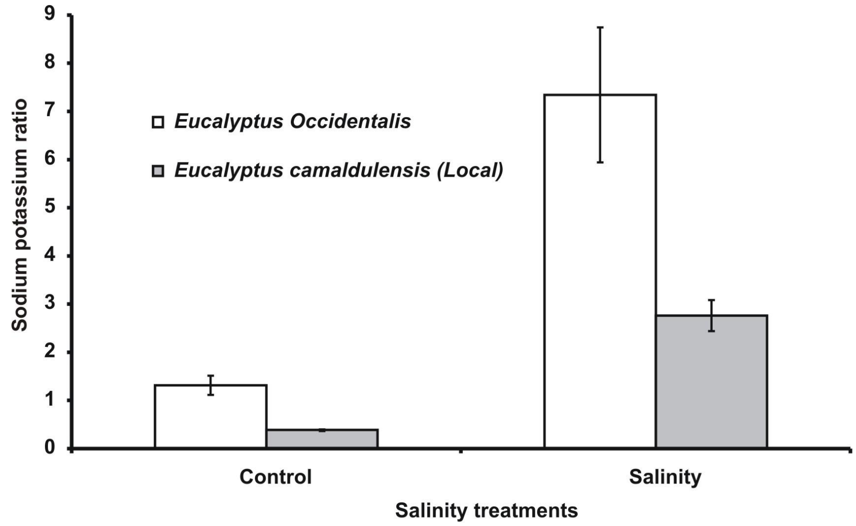

Fig. 4. Sodium:potassium ratio in leaves of Eucalaptus spececies grown with and without NaCl salinity in root medium

Absolute dry matter would also be used as a selection criterion for salinity tolerance of plants (Aslam *et al.*, 1993). Although *E. camaldulensis* (Silverton) responded significantly to applied salinity stress in terms of SDM reduction, however, its higher absolute biomass at salinity as well as at control treatment makes difficult to ignore this species. *E. campaspe* and *E. melliodora* were least efficient in dry matter production hence may not be selected for use of saline soils inspite of their more relative SDW.

E. occidentalis can be termed as saline tolerant, but its low absolute dry matter at normal as well as under saline conditions does not make this species favorite to grow under saline conditions. Whereas *E. camaldulensis* (local) produced significantly more SDM at both salinity levels, hence can be selected to grow on saline lands inspite of significant growth reduction under salinity treatment. Both of these species were further studied in a solution culture experiment to reconfirm their growth behavior and to study ionic composition of these species under saline conditions.

Growth behavior of *E. occidentalis* to salinity stress was not consistent in this experiment as indicated by significant reduction in growth parameters under salinity stress. This slight variation in growth performance may be due to the differences in environmental conditions (Maas, 1990). Aslam (1993) reported that a number of environmental factors including temperature and humidity determined growth behavior of plants under adverse soil conditions including salinity. The temperature ranged between 6.9 and 21.6 °C during experiment-I, while it ranged between 18.3 and 30.6°C during experiment-II. Increased temperature in second experiment aggravated the hazardous effects of salinity stress. Thus high temperature might has changed the salinity tolerance of *E. occidentalis* in terms of relative shoot and root dry weights.

Growth differences among both species were, however, consistent in experiment-II. The differences among both species were > 1.5 folds in PH, >4 folds in SDM and > 5 folds in RDM at both salinity levels. *E. camaldulensis* performed better for all of growth parameters than *E. occidentalis*.

Salt stress affects uptake, transport and utilization of different nutrients (Marschner, 1995; Grattan and Grieve, 1999; Zhu, 2003). This imbalance in nutrient uptake may result in excessive accumulation of Na^+ and Cl^- in tissue (Saqib *et al.*, 2000) and ultimately reduction in crop yield. Hence concentration of Na^+ in plants is a good indicator of salinity tolerance. Species differ greatly in their tolerance to salinity hence may differ in Na^+, K^+ and Cl^{-1} concentration in their tissues (Marcar *et al.*, 1995; Rawant and Banerjee, 1998; Saqib *et al.*, 2004; Munns, 2005).

In present experiment, salinity stress caused significant effect on Na^+, K^+ and Cl^{-1} uptake in both species. Sodium concentration in leaves of *E. camaldulensis* (Local) was significantly lower than in leaves of *E. occidentalis* at both salinity levels. Salt tolerance can be achieved by salt exclusion/inclusion. Different degrees of salt exclusion and inclusion among various species have been reported (Gorham *et al.*, 1985). *E. occidentalis* can be termed as Na^+ includers as inspite of high Na^+ concentration in its leaves, its relative biomass production was significantly more than *E. camaldulensis* at saline conditions (Table 1 & 2).

High concentration of Na^+ in root environment can restrict K^+ acquisition (Subbarao *et al.*, 1990; Liu *et al.*, 2001; Saqib *et al.*, 2004) ultimately affecting plant growth. Hence, adequate K^+ concentration in plant tissues is essential for survival particularly in saline soils. In present experiment, K^+ concentration was significantly decreased in *E. camaldulensis* (Salt sensitive) at high salinity (Fig. 2). High Na^+ concentration in root environment did not affect K^+ concentration in *E. occidentalis* as reduction in K^+ concentration was less than five percent only. This is another mechanism of salinity tolerance observed in *E. occidentalis*. Na:K ratio can also be used as an indicator of salinity tolerance in higher plants (Saqib *et al.*, 2005; Munns, 2005) as a low Na:K ratio in the cytosol is essential for normal cellular functions of plants (Chinnusamy, *et al.*, 2005).

Chloride concentration was higher in *E. occidentalis* at high salinity level and case was opposite under control treatment. It clearly suggested that *E. occidentalis* had some mechanism to accumulate more Na^+ and Cl^{-1} ions in its leaves and maintained its growth under salinity stress. This excessive entry of ions though facilitates to osmotic adjustment within plants but they have to pay the price in terms of growth reduction (Marcar, 1995). More biomass is a pre-requisite for any species to be selected for rehabilitation of waste saline lands. Hence, *E. camaldulensis* is a better option to reduce desertification of saline lands.

CONCLUSION

Species differ significantly for biomass production at both salinity levels in first screening experiment. Salinity stress significantly affected growth parameters in all species but to varying degrees. The selected species varied significantly for growth and ionic composition in second solution culture study. Relative biomass production was significantly more in *E. occidentalis*. Ion inclusion and maintenance of K in tissues are possible mechanisms of higher relative biomass in *E. occidentalis*. However, absolute growth performance of *E. camaldulensis* (Local) at control as well as under salinity stress makes this species desirable to be grown on saline lands. However, results of these solution culture experiments warrant further physiological studies to explore salinity tolerance mechanisms and these can be used for forest breeders to improve salinity tolerance in trees.

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