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Changes in growth and ionic composition of *Eucalyptus camaldulensis* under salinity and waterlogging stress; a lysimeter study

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Abstract

Response of salt tolerant eucalyptus species (Eucalyptus camaldulensis, Local) to dual stress of salinity and waterlogging was studied in a lysimeter. The three salinity levels viz S_1 (Control: 3.5 dS m^{-1}), S_2 (15 dS m^{-1}) and S_3 (30 dS m^{-1}) were developed in the lysimeters. Six month-old uniform saplings of E. camaldulensis were transplanted in each Lysimeter. Six weeks after transplanting, waterlogging (anaerobic stress) was imposed in half of the lysimeters and maintained continuously for 12 weeks. Plants were harvested after 22 weeks of transplanting. Salinity significantly ($p<0.01$) depressed all of the growth parameters including tree height, number of branches and plant biomass compared to non-saline treatment. Waterlogging alone did not affected growth parameters significantly. Hazardous effects of salinity were many folds aggravated when plants were subjected to waterlogging. Different aged leaves exhibited significant variations for sodium (Na^+) and chloride (Cl^-) concentrations. Na^+ and Cl^- concentration in leaves were significantly increased when plants were subjected to dual stress of salinity and waterlogging compared to salinity and waterlogging alone. Salinity stress significantly decreased potassium (K) concentration and K^+:Na^+ ratio in leaves of eucalyptus. Concentration of both Na^+ and Cl^- concentration was significantly more in old leaves compared to medium and young leaves indicating an adoption of E. camaldulensis against salinity stress.

Key words: Eucalyptus, salinity, Na^+, Cl^-, waterlogging, ionic composition

Introduction

Soil desertification/degradation resulting from ever increasing salinity and sodicity is a serious threat to agricultural productivity and sustainability (Qadir *et al*., 2006). The problem of salinization is seriously increasing in arid and semi-arid regions around the globe, particularly in irrigated agriculture (Cheraghi, 2004). About 20% of irrigated soils around the globe (more than 75 countries) are affected by excess of salts within root zone (Qadir *et al*., 2007). More than 6.3 million hectares of agricultural land in Pakistan is affected by salinity to varying degrees (Ghafoor *et al.,* 2004). Salinity affects plant growth mainly through osmotic stress, ionic imbalances and specific ion toxicity (Grattan and Grieve, 1999; Saqib *et al.,* 2004, 2005; Munns, 2005; Rezaei *et al.,* 2006; Tahir *et al.,* 2006).

In addition to these chemical changes, certain physical problems also limit plant growth in salt affected soils. Qadir *et al.* (2007) has elaborated these structural problems in salt affected soils including slaking, swelling and dispersion of clay, surface crusting and hard setting resulting in poor water and air movement. Hence sodic soils have low infiltration and percolation of water, may act as waterlogged soils if heavily irrigated without adding some kind of amendments (Gypsum) as a source of calcium (Ca) (Qadir *et al.,* 2005, 2007). Waterlogging results in a change of mode of respiration from aerobic to anaerobic because plant roots face oxygen deficiency under waterlogged conditions (Marschner, 1995). Thus low energy production in roots because of anaerobic respiration disturbs the nutrient and water uptake by plants (Jackson, 1979; Morard and Silvestre, 1996). This low energy production by plants under waterlogged conditions may affect sodium exclusion from plant roots, which is major salinity tolerance mechanism in many glycophytes (Marschner, 1995; Saqib *et al*., 2005). Hence, interactive effect of salinity and waterlogging may affect more than caused by salinity and waterlogging alone (Qureshi and Barrett-Lennard, 1998; Saqib *et al.,* 2004).

The effect of dual stress of salinity and waterlogging on various crop species has been reported by a number of researchers (Akhtar *et al*., 1994, 1998; Saqib *et al.,* 2004). However, comparatively little information is available on the responses of woody tree species to the combination of salinity and waterlogging stresses. Moreover, most of the studies on interactive effect of salinity and waterlogging stress on crops and tree species were conducted in the controlled environment, mostly in the hydroponics systems and for short duration. Hence, there is a dire need to study response of plants to salinity and waterlogging under field conditions. Studies in lysimeter resemble to soil conditions and may be conducted on long term basis because of large

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volume of soil. In a preliminary study, *Eucalyptus camaldulensis* performed better than many other eucalyptus species under salt stress in solution culture. The present experiment was conducted in a lysimeter for 22 weeks to study growth performance of *Eucalyptus camaldulensis* under salinity and waterlogging stresses.

Materials and Methods

The experiment was conducted in a rain-protected wire house at the Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad. The average day and night temperatures during the study were 32 and 20 °C, respectively.

The study was conducted twice in specially designed square shaped concrete lysimeters (lxl m^2) having 30 cm layer of coarse sand at bottom, a thin layer of glass wool in between sand and a soil column of 133 cm. The total profile (sand + soil) was of 165 cm. Three salinity levels used in the study were: S_1 (control: 3.5 dS m^{-1}), S_2 (15 dS m^{-1}) and S_3 (30 dS m^{-1}). Salinity was developed by saturating the soil with salinized water (NaCl was dissolved in calculated amount of canal water). Half amount of water was applied from surface and the other half from bottom of the lysimeter through provisions of inlet and outlet holes.

After attaining desired salinity levels, six uniform 6-month-old saplings of *Eucalyptus camaldulensis* (Local) were transplanted in each Lysimeter. Six weeks after transplanting, plants were thinned to 4. At this stage waterlogging/anaerobic stress (in half of the lysimeters) was imposed and maintained continuously for 12 weeks. However, soil remained in saturated conditions for another 4 weeks. Experiment was laid out in completely randomized design in factorial arrangements with four repeats.

Twenty two weeks after transplanting, plant height was measured and numbers of branches per plant were counted. Leaves from old (the lower most two branches from base of the tree), middle (branches exactly falling in the center of upper and lower most branches) and young branches (two upper most branches) of the each plant were collected in paper bags. After separating leaves, plants were harvested by cutting at ground level. The fresh weight of twigs and leaves was recorded immediately after harvest. The leaves were then washed with distilled water and dried at 70°C in a forced air driven oven for 48 h. The oven dried leave samples were fine ground in a wily mill to pass through 1 mm sieve. The fine ground leave samples (1 g) were digested in tri-acid mixture (sulfuric acid, nitric acid and perchloric acid) (Miller, 1998). Potassium and Na^+ were determined on a flame photometer (Jenway PFP-7). For chloride (Cl^{-1}) determination, plant samples were extracted with HNO_3 and chloride was determined from this extract using chloride analyzer (Corning Chloride Analyzer 926). The data were analyzed statistically following the methods of Gomez and Gomez (1984) using MStat-C (Michigan State University, 1996). The significance of differences among the means was compared using standard error computed as $s/\sqrt{n}$, where s is the standard deviation and n shows the number of observations.

Results and Discussion

Growth parameters

Salinity and waterlogging stresses significantly reduced all of the growth parameters significantly (Figures 1-4). In most plants growth gradually reduces as salinity increases above a threshold value which varies in different species (Saqib *et al.,* 2004, 2005; Tahir *et al.,* 2006). Mean tree height of *Eucalyptus camaldulensis* decreased significantly as salinity increased in the root medium at waterlogging as well as non-waterlogging treatment (Figure 1). Similar reduction in tree height was observed in Eucalyptus in a solution culture experiment (Nasim *et al.,* 2008). Effect of waterlogging on tree height was non-significant at control (3.5 dS m^{-1}) and S_2 treatment (15 d Sm^{-1}) however, waterlogging significantly depressed tree height at S_3 (30 dS m^{-1}). The reduction in mean tree height was 1.5% due to waterlogging, 7.6% due to S_2, 10% due to combined effect of S_2 and waterlogging, 45.5% due to S_3 and 59.2% due to combined effect of S_3 and waterlogging.

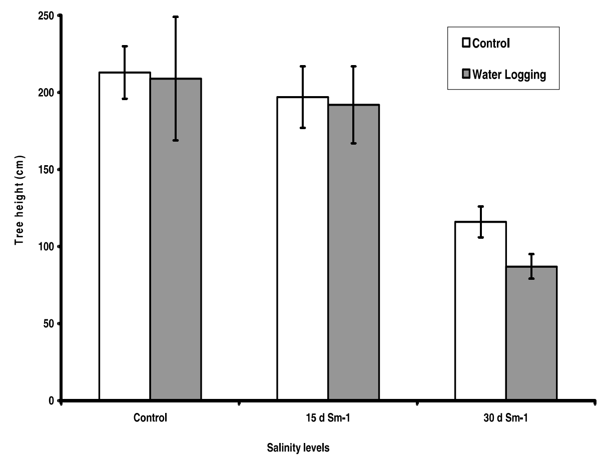

Fig. 1. Effect of salinity and waterlogging on tree height of eucalyptus camaldulensis

There was significant main and interactive effect of salinity and waterlogging on number of branches (NB) per plant (Figure 2). Salinity in the root medium significantly reduced number of branches (NB) per plant. Maximum NB (40) was observed at S_1, while it was lowest at S_3 (EC 30 dS m^{-1}) both at waterlogging and non-waterlogging conditions. Waterlogging alone did not affect NB at control treatment, however, it reduced NB when combined with S_2 (15 dS m^{-1}) and S_3 (30 dS m^{-1}) treatment (Figure 2). Number of branches was about 47% lower at S_3 and waterlogging treatment compared to only S_3.

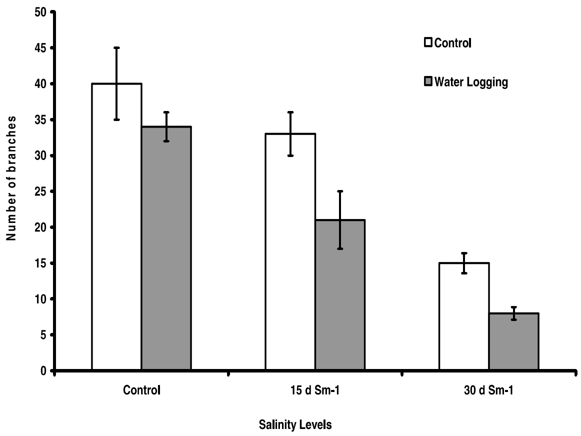

Fig. 2. Effect of salinity and water logging on number of branches of *Eucalyptus camaldulensis*

There was significant main and interactive effect of salinity and waterlogging on fresh weight of twigs (FWT). Both stresses in soil significantly depressed the mean fresh weight of twigs of *Eucalyptus camaldulensis* (Figure 3). Fresh weight of twigs in plants was more than 5 times lower at S_3 (30 dS m^{-1}) treatment compared to non-waterlogging control. Reduction in FWT was increased as salinity was increased in the root medium. Waterlogging alone did not affect FWT at control treatment, however, it reduced FWT at S_2 (15 dS m^{-1}) and S_3 (30 dS m^{-1}) treatment. Reduction in FWT due to salinity was aggravated significantly as plants were affected by waterlogging. The relative reduction in fresh weight of twigs was 33.9, 56.8, 79.2 and 89.5% in the case of S_2 (15 dS m^{-1}) alone, S_2 (15 dS m^{-1}) and waterlogging, S_3 (30 dS m^{-1}) alone and S_3 and waterlogging, respectively.

There was significant main and interactive effect on fresh weight of leaves (FWL) of Eucalyptus (Figure 4). Fresh weight of leaves of *Eucalyptus camaldulensis* was significantly reduced as salinity was increased in the root medium. Fresh weight of leaves was about 75% and 30% at S_2 (15 dS m^{-1}) and S_3 (30 dS m^{-1}) levels, respectively as compared to non-waterlogging control. Waterlogging alone did not affect FWL of eucalyptus; however, it aggravated the reduction in FWL due to salinity at 15 dS m^{-1} and 30 dS m^{-1}. The minimum fresh weight of leaves was recorded under the combined stress of high salinity + waterlogging. The relative reduction in fresh weight of leaves varied from 2.2 (waterlogging alone) to 92.2% (waterlogging with 30 dS m^{-1}salinity). Many of earlier scientists also reported significant reduction in biomass under salt stress in eucalyptus (Qureshi *et al.,* 1993; Marcar *et al.,* 1995).

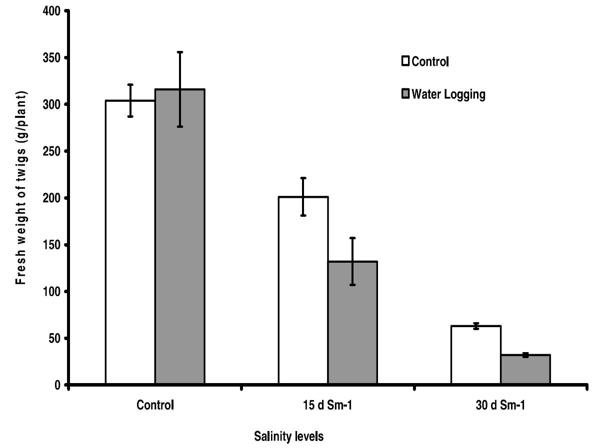

Fig. 3. Effect of salinity and waterlogging on fresh weight of twigs of Eucalyptus camaldulensis

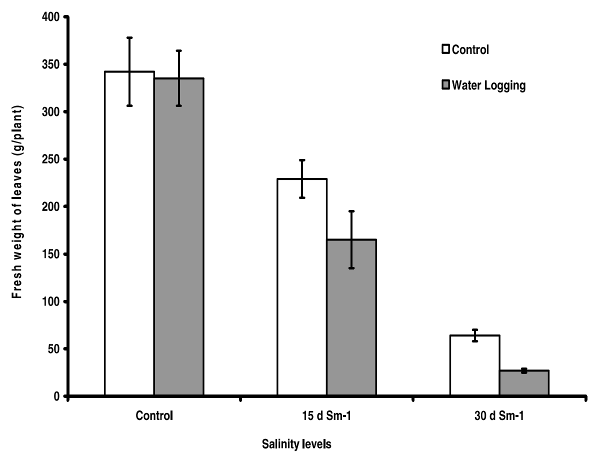

Fig. 4. Effect of salinty and waterlogging on fresh weight of leaves of *Eucalyptus camaldulensis*

Physiological parameters

Tissue ion concentrations are the result of ion transport and growth of shoot. The balance between water and salt uptake could be maintained by reducing transpiration but at the expense of reduced carbon fixation and reduced growth rate (Marschner, 1995). The rate of ion transport depends not only on the rate of shoot growth but also on the external concentration of salts. Salt stress affects uptake, transport and utilization of different nutrients (Marschner, 1995; Grattan and Grieve, 1999; Zhu, 2003). The imbalance in nutrient uptake under salinity stress may result in excessive accumulation of Na^+ and Cl^- in tissue (Saqib *et al.,* 2004; Tahir *et al.,* 2006) and ultimately reduction in crop yield.

Hence concentration of Na^+ in plants is a good indicator of salinity tolerance.

There was significant main and interactive affect of salinity and waterlogging on Na^+ concentration in lower, middle and upper leaves of *Eucalyptus camaldulensis* (Table 1). Increased salinity in the root medium, significantly increased Na^+ concentration (>2 folds) in leaves of *Eucalyptus* compared to control (Table 1). Na^+ was significantly lower in young leaves at all salinity treatments at non-waterlogging conditions. Waterlogging alone did not increase mean Na^+ concentration in leaves as compared to control, but waterlogging + salinity resulted in a significant increase (2.5-4.5 folds) in the Na^+ concentration of leaves from different branches. The accumulation of Na^+ was the highest (632 mmole Na kg^{-1}) at S_3 and waterlogging followed by S_2 and waterlogging (321 mmole Na kg^{-1}) and S_3 (295 mmol kg^{-1}). Interactive effect of salinity X waterlogging clearly showed that accumulation of Na^+ in leaves increased when the plants were exposed to dual stress of waterlogging and salinity (30 dS m^{-1}). Na^+ in lower leaves was maximum at all salinity treatment compared to other leaves. Saqib *et al.* (2004) and Tahir *et al.* (2006) also reported significant increase in Na^+ concentration in leaves of different wheat cultivars under salinity stress and reported a significant reduction in growth of plants with an increase in Na^+ concentration in leaves. In our preliminary solution culture experiments, different eucalyptus species accumulated higher amounts of Na in their leaves under salinity treatment (Nasim *et al*., 2008).

There were significant main and interactive effect of salinity and waterlogging on $C1^-$ concentration in leaves (Table 1). Salinity enhanced $C1^-$ accumulation significantly ($p<0.05$) in leaves of *Eucalyptus camaldulensis* compared with control and waterlogging treatment. Waterlogging alone did not have an effect on Cl^- concentration, but combined stress of salinity andwaterlogging resulted in marked increase in Cl^- concentration (> 2 folds). The highest Cl^- concentration was observed under S_3 alone and S_3 and waterlogging. Lowest leaf Cl^- concentration was found in control and waterlogging alone. Saqib *et al.* (2004) also reported an increase in Cl^- concentration under salinity stress. Chloride concentration was significantly lower in upper leaves at all treatments compared to middle and lower leaves.

Adequate K^+ concentration in plant tissues is essential for survival particularly in saline soils (Marschner, 1995). High concentration of Na^+ in root environment can restrict K^+ acquisition (Subbarao *et al.,* 1990; Liu *et al.,* 2001; Saqib *et al.,* 2004) leading to inhibition of K requiring processes in the cytoplasm. Nutritional imbalances as a growth limiting factor under saline and /or waterlogging conditions has also been stressed by Grattan and Grieve (1999) and Tahir *et al.* (2006). Reduced K concentration in leaves under salinity stress in present study also revealed that poor growth of plants in saline and waterlogging condition may not only due to decreased water potential (osmotic effect) but also due to ionic imbalances particularly of Na^+, Cl^- and K^+. There was significant main and interactive effect of salinity and waterlogging on K^+ concentration in leaves of eucalyptus (Table 2)

Table 1. Leaf sodium (Na^+) and chloride (Cl^-) concentration in *Eucalyptus camaldulensis* grown with salinity and waterlogging

Parameters	Branches	Non-waterlogging			Waterlogging		
		Control	15 dS m^{-1}	30 dS m^{-1}	Control	15 dS m^{-1}	30 dS m^{-1}
Na^+	Old	151 ± 9	334 ± 24	295 ± 14	199 ± 5	321 ± 21	632 ± 20
(mmole kg^{-1})	Middle	100 ± 10	234 ± 13	250 ± 17	125 ± 8	274 ± 15	495 ± 21
	Young	92 ± 5	145 ± 14	181 ± 19	110 ± 11	222 ± 16	375 ± 15
Cl^-	Old	130 ± 14	193 ± 6	283 ± 7	65 ± 3	161 ± 11	395 ± 11
(mmole kg^{-1})	Middle	122 ± 12	182 ± 12	232 ± 11	74 ± 6	149 ± 8	282 ± 20
	Young	85 ± 5	125 ± 6	197 ± 12	89 ± 4	156 ± 5	334 ± 13

Table 2. Leaf potassium (K^+) and K^+:Na^+ ratio in *Eucalyptus camaldulensis* grown with salinity and waterlogging

Parameters	Branches	Non-waterlogging			Waterlogging		
		Control	15 dS m^{-1}	30 dS m^{-1}	Control	15 dS m^{-1}	30 dS m^{-1}
K^+	Old	275	173	187	171	178	148
(mmole kg^{-1})	Middle	443	411	378	261	294	223
	Young	462	445	395	342	374	327
K^+:Na^+ ratio	Old	1.82	0.52	0.63	0.86	0.55	0.23
	Middle	4.43	1.76	1.51	2.09	1.07	0.45
	Young	5.02	3.07	2.18	3.11	1.68	0.87

Overall treatment comparison revealed that waterlogging alone and as well as along with high salinity significantly ($p<0.01$) depressed K^+ accumulation in leaves compared with control. Saqib *et al*. (2004) and Tahir *et al*. (2004) also reported significant reduction in K^+ concentration in leaves of plants grown with salinity in hydroponics experiments. Significant differences were found in the concentration of K^+ in leaves belonging to different aged branches. The leaves of young branches maintained high concentration of K^+ whereas it was the poorest in the case of leaves on old branches. *Eucalyptus camaldulensis* (Local) accumulated less Na^+ and Cl^- and more K^+ in young leaves than older ones. One adaptation to salinity and other stresses including waterlogging in plants, is to rely on the youngest leaves for photosynthetic CO_2 assimilation.

Conclusion

Salinity significantly depressed all of the growth parameters in *E. camaldulensis*. Waterlogging alone did not affected growth parameters; however, in combination with salinity it significantly affected growth parameters and ionic composition of leaves of *E. camaldulensis* (local). Na^+ and Cl^- concentration was significantly more in old leaves indicating an adoption of *E. camaldulensis* against salinity stress. Na^+ and Cl^- concentration in leaves were significantly increased when plants were subjected to dual stress of salinity and waterlogging compared to salinity and waterlogging alone.

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