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ION PARTITIONING, K/Ca AND K/Na RATIOS OF *EUCALYPTUS CAMALDULENSIS* GROWN UNDER NaCl SALINITY

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Abstract

Effect of NaCl salinity on growth, ion partitioning, K/Ca and K/Na ratios of four provenances of *Eucalyptus camaldulensis* was studied under 5 salinity levels i.e. 0, 0.5, 1.0, 1.5 and 2.0 % NaCl with five replications. It was noted that plant height, stem diameter, shoot and root fresh and dry weights were reduced in most of the provenances with the increase in NaCl concentration. However, reduction in growth was much prominent in Provenance-I and Provenance-IV. Accumulation of Na^+ ions increased with increase in salinity levels in root and shoot in all the provenances under study. However, Provenance-II showed least accumulation. The concentration of K^+ and Ca^{2+} decreased both in shoot and root of all the provenances due to applied NaCl. The Provenance-II maintained the highest concentration of K^+ and Ca^{2+} both in shoot and root when exposed to NaCl salinity. Sodium chloride treatment increased the K/Ca ratio in shoot but not in root. At 2% NaCl concentration K/Ca ratio was maximum in shoot and minimum in root of all the provenances. K/Na ratio decreased both in shoot and root with the increase in NaCl concentration. At 2% NaCl concentration, however, provenance II maintained the highest K/Na ratio. It appears from the studies that *Eucalyptus* provenances having high K^+ and Ca^{2+} contents may suppress the toxic effects of Na^+ and hence could be recommended to be grown on highly salt-affected soils.

Introduction

Soil salinity influences the integrity of cell membrane by inducing change in structure and composition of membrane proteins and affects both chemical and physiological properties of the cell wall. High sodium concentration relative to other salts can disrupt root permeability to ions by displacing calcium in the plasma membrane (Rengel, 1992). Secondary effects may be caused by upsetting calcium metabolism and uptake of essential nutrients such as potassium (Ashraf & Sarwar, 2002; Gorham & Wyn Jones, 1993). Plant exposed to saline environment may overcome excess of ions in the root medium through different physiological traits such as discriminatory transport, which either minimizes or removes Na^+ and Cl^- from the xylem (Greenway & Munns, 1980), compartmentation at cellular, tissue and organ levels (Hussain *et al*., 2002; Gorham & Wyn Jones 1993), synthesis and accumulation of compatible solutes in the cytoplasm (Greenway & Munns; 1980, Gorham *et al*., 1995). Sodium is accumulated under saline conditions (Glenn *et al*., 1996) and accumulation of large amounts of ions (40% of dry weight) also takes place in subtropical perennial halophyte (*Atriplex griffithii*) (Khan *et al.*, 2000).

Eucalyptus camaldulensis has been identified as a tree species tolerant to salinity and water logging, and has more than 85% survival rate under moderately saline soil conditions (Qureshi *et al.*, 1988). Hence, it is the most successful tree species under a variety of saline conditions (Qureshi *et al*., 1993; Marcar, 1993; Pearce *et al*., 1990; Clemen & Pearson, 1997). However, information is scanty on the partitioning of potassium and calcium and their relation with sodium in *Eucalyptus* when exposed to saline environment.

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Materials and Methods

A gravel culture experiment was conducted to study the influence of NaCl salinity on growth, ion partitioning, K/Ca and K/Na ratio in four provenances of *Eucalyptus camaldulensis*. Experiment was carried out in plastic pots, measuring 22 x 20 cm and filled with washed sterilized gravels which was placed in another bigger plastic pot measuring 25 x 23 cm having no hole, in order to collect drained solution from the pots filled with gravel. The pots with gravels were filled with ½ strength Hoagland's solution (Hoagland & Arnon, 1950). Three months old seedlings of *Eucalyptus* comprising provenances I, II, III and IV were transplanted and allowed to grow for 15 days. Four salinity treatments, (Control, 0.5, 1.0, 1.5 and 2.0%) were created with NaCl. Each treatment had 5 replications. For appropriate growth, the pH of solution was checked daily, maintained to 6.5 using dilute solution of H_2 SO_4. The solutions were changed weekly and plants were allowed to grow for 1 month and then harvested. After harvesting their shoot length, root length, shoot and root fresh and dry weight and diameter of the stem were measured.

The leaves of *Eucalyptus* were dried in an oven at 70 ± 2^{o}C for 72 h and were ground in a Willey micro mill, so as to pass through 2 mm sieve. The ground material was digested with sulphuric acid and hydrogen peroxide according to the method of Wolf (1982) for the determination of ionic contents.

The concentrations of sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) were analyzed by flame photometer (Jenway PFP-7). A graded series of standard (ranging from 10-100 ppm) of Na^+, K^+ and Ca^{2+} were compared for standard curve and total quantities in samples were calculated. The K/Na and Ca/Na ratios were calculated and all the collected data were subjected to statistical analyses, using analysis of variance technique and Duncan's New Multiple Range test was applied to compare the means (Steel & Torrie, 1980).

Results

Growth: Plant height was significantly influenced by NaCl treatments but no significant differences in the plant height were observed among different provenances of *Eucalyptus camaldulensis* except provenance I (Table 1). The differences among provenances were prominent only at 2% NaCl. Provenance II performed better under all salinity levels than other provenances. Similarly at 2% NaCl concentration, stem diameter was minimum in all the provenances. Increasing concentration of NaCl showed decrease in fresh weight of shoot and root. At 2% NaCl concentration, fresh weight of shoot and root was minimum. The decrease was greater (61%) in root as compared to shoot (41%). Fresh weight of shoot was maximum in provenance II and it was minimum in provenance IV. In case of root, the least fresh weight was observed in provenance I and provenance III.

Dry weight of shoot and root revealed no significant difference in different provenances except provenance II which showed significant difference (Table 1). Increasing concentration of NaCl showed inhibitory effect on dry weight of shoot and root. At 2% NaCl treatment, dry weight of shoot and root showed maximum decrease. Provenance II performed better at all salinity treatments.

Table 1. Effect of NaCl induced salinity on the growth of *Eucalyptus camaldulensis*.

Provenances	Treatments (T) with NaCl (%)					
	(T1) 0	(T2) 0.5	(T3) 1.0	(T4) 1.5	(T5) 2.0	Mean
	Height of plants at harvest (cm)					
Provenance I	51.0cd	51.5c	48.5de	45.8f	42.0g	47.7b
Provenance II	55.0a	54.8a	51.8bc	47.0ef	42.8g	50.3a
Provenance III	55.1a	54.3a	51.6c	46.0f	41.2g	49.6a
Provenance IV	55.6a	54a b	50.8cd	45.6f	40.6g	49.3a
Mean	54.0a	53.6a	50.6b	46.0c	41.6d	
	Stem diameter of plant at harvest (cm)					
Provenance I	3.2a	2.7abcd	2.6bcde	2.1efg	1.26h	2.39a
Provenance II	3.0abc	2.8abcd	2.6bcde	2.4def	1.3h	2.41a
Provenance III	3.1ab	2.7abcd	2.5cdef	2.3defg	1.28g	2.48a
Provenance IV	3.0abc	2.6bcde	2.4def	2.0fg	1.2h	2.24a
Mean	3.0a	2.7b	2.5b	2.2c	1.4d	
	Fresh weight of shoot (g)					
Provenance I	18.0a b	15.3cde	14.8def	13.1fgh	10.0i	14.2b
Provenance II	17.0abc	16.5bcd	15.5cde	14.4defg	12.7gh	15.2a
Provenance III	17.0abc	16.1bcd	15.0cdef	13.9efgh	10.5i	14.5ab
Provenance IV	18.6a	15.5cde	14.7def	12.4h	8.7i	13.9b
Mean	17.7a	15.9b	15.0b	13.4c	10.5d	
	Fresh weight of root (g)					
Provenance I	4.5ab	3.0ef	2.7fg	1.8ij	1.6j	2.7b
Provenance II	4.3b	3.6c	3.4cd	2.6g	2.1hi	3.2a
Provenance III	3.4cd	3.2de	2.9efg	2.2h	1.8ij	2.7b
Provenance IV	4.8a	2.9efg	2.6g	1.7j	1.1k	2.6b
Mean	4.3a	3.2b	2.9c	2.1d	1.7e	
	Dry weight of shoot (g)					
Provenance I	5.4ab	4.5cde	3.8efg	3.0gh	2.5h	3.8bc
Provenance II	5.1abc	4.8bcd	4.5cde	3.7efg	3.3fgh	4.3a
Provenance III	5.9a	4.7bcd	4.1def	2.6h	2.8h	3.9ab
Provenance IV	5.8a	4.5cde	3.6fg	2.5h	1.5i	3.6c
Mean	5.6a	4.6b	3.9c	2.9d	2.5e	
	Dry weight of root (g)					
Provenance I	2.4a	1.7cd	1.4e	1.2ef	0.8g	1.5b
Provenance II	2.2ab	2.0bc	1.7cd	1.4e	1.2ef	1.7a
Provenance III	2.0bc	1.9c	1.5de	1.3ef	0.8g	1.5b
Provenance IV	2.5a	1.7cd	1.3ef	1.0fg	0.5h	1.4b
Mean	2.3a	1.8b	1.5c	1.2d	0.81e	

Selectivity of ion uptake: Na^+ concentration was significantly increased by NaCl treatments being maximum at 2% NaCl (Table 2). The mean value for provenances indicated that the highest Na^+ concentration in shoot was recorded in provenance IV followed by provenance I, III and II. There were significant differences among provenances regarding K^+ concentration. Treatment with NaCl significantly decreased the K^+ concentration in shoot and root. At NaCl concentration of 1.5%, K^+ concentration

was maximum in provenance II and minimum in provenance I (shoot) and IV (root). At 2% NaCl, mean concentration of K^+ was minimum and the treatments mean differed significantly both in shoot and root. Maximum K^+ concentration both in shoot as well as in root were observed in provenance II followed by III, I and IV, respectively.

Table 2. Concentration of ions in root and shoot of different provenances of *Eucalyptus camaldulensis* grown under NaCl induced salinity.

Provenances	Treatments (T) with NaCl (%)					
	(T1) 0	(T2) 0.5	(T3) 1.0	(T4) 1.5	(T5) 2.0	Mean
	Effect of NaCl on Na^+ concentration in shoot (ppm)					
Provenance I	1498h	2496fg	2645e	2870d	3585b	2618b
Provenance II	1566h	2422g	2518f	2804d	3445c	2557c
Provenance III	1570h	2465fg	2533f	2818d	3568b	2590bc
Provenance IV	1576h	2502fg	2658e	2894d	3695a	2661a
Mean	1552e	2471d	2582c	2893b	3573a	
	Effect of NaCl on Na^+ concentration in root (ppm)					
Provenance I	1200i	1539g	1714de	1828c	968a	1651a
Provenance II	1260h	1491g	1615f	1732de	1829c	1585c
Provenance III	1279h	1500g	1674e	1745d	1901b	1621b
Provenance IV	1268h	1547g	1725de	1840c	1997a	1674a
Mean	1251e	1522d	1682c	1788b	1922a	
	Effect of NaCl on K^+ concentration in shoot (ppm)					
Provenance I	3839b	3655d	3410g	3124k	3085i	3428c
Provenance II	3930a	3847b	3674d	3278i	3082i	3562a
Provenance III	3638d	3800c	3568e	3208j	3083i	3464b
Provenance IV	3552f	3531f	3345h	3156k	3087i	3333d
Mean	3739a	3708b	3505c	3194d	3087e	
	Effect of NaCl on K^+ concentration in root (ppm)					
Provenance I	3578a	2063d	1698gh	542j	434k	1675b
Provenance II	3610a	2157b	1737f	610i	530j	1727a
Provenance III	3595a	2105c	1725fg	536j	478k	1687b
Provenance IV	3609a	1978e	1692h	444l	337m	1611c
Mean	3598	2076b	1713c	533d	445e	
	Effect of NaCl on Ca^{2+} concentration in shoot (ppm)					
Provenance I	3191a	2635efg	2600efg	2337h	1856i	2519ab
Provenance II	3125ab	3002abc	2662def	2449fgh	1799i	2599a
Provenance III	3208bc	2898cd	2625efg	2411gh	1804i	2522ab
Provenance IV	3129a	2326h	2677de	2369h	1860i	2470b
Mean	3077a	2698b	2644b	2391c	1829d	
	Effect of NaCl on Ca^{2+} concentration in root (ppm)					
Provenance I	5532b	3210 e	2618i	2362l	2018o	3148c
Provenance II	5590a	3372c	2720g	2685h	2355l	3344a
Provenance III	5548b	3316d	2637i	2465k	2243 m	3241b
Provenance IV	5593a	3165f	2502j	2078n	1921p	3051d
Mean	5565a	3265b	2619c	2396d	2134e	

Table 3. K/Ca and K/Na ratios in different provenances of *Eucalyptus camaldulensis* grown under NaCl induced salinity.

Provenances	Treatments (T) with NaCl (%)					
	(T1) 0	(T2) 0.5	(T3) 1.0	(T4) 1.5	(T5) 2.0	Mean
	Effect of NaCl on K/Ca ratio in shoot					
Provenance I	1.20	1.39	1.31	1.33	1.67	1.36
Provenance II	1.27	1.28	1.38	1.34	1.71	1.40
Provenance III	1.25	1.33	1.37	1.33	1.71	1.40
Provenance IV	1.14	1.52	1.25	1.33	1.66	1.38
Mean	1.22	1.37	1.33	1.34	1.69	
	Effect of NaCl on K/Ca ratio in root					
Provenance I	0.65	0.64	0.65	0.23	0.24	0.53
Provenance II	0.65	0.64	0.64	0.23	0.23	0.52
Provenance III	0.65	0.63	0.65	0.22	0.21	0.52
Provenance IV	0.65	0.63	0.68	0.21	0.17	0.52
Mean	0.65	0.64	0.66	0.22	0.22	
	Effect of NaCl on K/Na ratio in shoot					
Provenance I	2.56	1.46	1.29	1.09	0.86	1.31
Provenance II	2.51	1.59	1.46	1.17	0.86	1.39
Provenance III	2.32	1.55	1.41	1.14	0.86	1.34
Provenance IV	2.25	1.41	1.26	1.09	0.87	1.25
Mean	2.41	1.50	1.36	1.10	0.86	
	Effect of NaCl on K/Na ratio in root					
Provenance I	2.98	1.34	0.99	0.30	0.22	1.10
Provenance II	2.87	1.45	1.08	0.35	0.29	1.09
Provenance III	2.81	1.40	1.03	0.31	0.25	1.04
Provenance IV	2.82	1.28	0.98	0.24	0.17	0.96
Mean	2.88	1.36	1.02	0.30	0.24	

Co-efficient of variation = 2.85%, S.E. for Treatments =0.404, S.E. for Provenances = 0.362
All such means which share a common English letter are not significantly different at least at $p<0.05$ according to DMR test.

The concentration of Ca^{2+} was significantly influenced by NaCl treatments both in shoot and root (Table 2). At 0.5% NaCl the Ca^{2+} concentration in provenance II, was significantly higher as compared to provenance I and IV in shoot, and provenances I, II, III in root. Increasing concentration of NaCl treatment showed decreasing trend in Ca^{2+} concentration of shoot and root, being the least in all the provenances at 2% NaCl concentration. At 0.5, 1.5 and 2% NaCl treatment, the roots of all the provenances differed significantly for Ca^{2+}. Maximum concentration of Ca^{2+} was recorded in provenance II and minimum in provenance IV.

K/Ca and K/Na ratios: K/Ca ratio (Table 3) showed marked difference in different provenances. NaCl increased the K/Ca ratio in shoot but not in root. At 2% NaCl concentration, K/Ca ratio was maximum in shoot and minimum in root of all the provenances. Maximum K/Ca ratio in shoot was observed at 2% NaCl concentration and minimum at 0.5% NaCl over control but their was no marked difference among the treatments from 0.5 to 1.5% NaCl concentration. Similarly, data regarding root revealed

that control plants and those treated with 0.5 and 1% NaCl treatments maintained the highest K/Ca ratio in root.

There was significant difference in the K/Na ratio of different provenances both in shoot and root (Table 3). At 2% NaCl concentration, K/Na ratio was lowest in shoot and root of all the provenances. Minimum K/Na ratio in shoot was observed at 2% NaCl concentration and maximum in control. The ratio decreased with the increase in NaCl treatments. Provenance II maintained the highest and provenance IV exhibited the least. Similarly, K/Na ratio in root revealed that control, 0.5 and 1% NaCl treatments maintained the highest ratio. The differences among treatments (from 1 to 2% NaCl concentrations) were marked but among the provenances it was about similar.

Discussions

NaCl showed significant reduction in the plant height and fresh and dry weight in all the provenances of *Eucalyptus.* This decrease was more prominent at the highest (2%) concentration of NaCl. The four provenances can be grouped on the basis of their growth performance as Provenance II and III being tolerant and Provenance I and IV as sensitive. Growth reduction under stressed condition has been reported by many workers (Aslam *et al*., 1993a, Lin & Kao 2001, Shereen *et al*., 2001, Arshi *et al*., 2002) in crops as well as in tree plants. The reduction in plant height under salinity may be due to higher concentration of salt which reduce the cell turgor potential (Ashraf *et al*., 1998; Qureshi *et al*., 2000) as the turgor potential is directly involved in cell elongation and cell division (Qureshi *et al*., 2000; Ashraf & Sarwar, 2002). The sensitivity of Provenance I and IV appears to be related to their turgor potential and water potential (Table 1). The growth of plant depends upon the various physiological processes like nutrient uptake, water absorption and transpiration. All these processes are adversely affected under saline condition (Mass, 1993, Aslam., 1998; Shereen *et al*., 2001; Qasim *et al*., 2003). Moezel & Bell, (1990) also reported that growth of *Eucalyptus camaldulensis* was reduced under saline condition.

As *Eucalyptus* is tolerant to salinity (Hussain *et al.,* 1991) at later stage of plant growth, so most of the provenances performed better upto 1.5% NaCl and reduction in biomass was less than 50% upto this level. At 2% NaCl, Provenance II had maximum fresh weight and dry weight of shoot and root while it was minimum in provenance IV. The maintenance of fresh weight of shoot and root in provenance II may be due to the maintenance of relative water content while the other provenances failed to do so (Minhas *et al*., 1997). The higher dry weight of shoot and root in provenance II may be due to the higher accumulation of nutrient and some organic solutes (Ashraf & Khan, 1994; Lacan & Durand, 1995) and assimilates (Leung & Giraudar, 1998; Netting 2000). Jaenicke *et al*., (1996) and Mansour *et al.,* (2002) indicated that salinity retarded the absorption of some macro and micro nutrients, necessary for proper development of plants. Reports in the literature also confirm that salinity retard some metabolic activities, like enzyme activities due to excessive Na^+, Cl^- or water deficiency in the root media (Ashraf & Sarwar, 2002). The provenance IV had the highest concentration of Na^+ in root and shoot but the rate of growth was minimum (Table 1), indicating negative correlation of growth and Na^+ concentration in the provenance II and III, maintaining low Na^+ concentration and higher growth rate. Present study indicated that Na^+ concentration increased with increase in NaCl concentration being maximum at the highest salinity level. Similar observation was recorded by Aslam *et al*., (1998) and Arshi (2002) in *Cassia angustifolia*.

The Na^+ absorbed may have been transported to the shoot resulting in comparatively low concentration of Na^+ in root than that of shoot. But provenance I and IV had higher concentration of Na^+ in root. Perhaps accumulation of Na^+ in root affects the cell permeability as a result of which cell elongation/cell division is adversely affected (Ashraf & Sarwar, 2002; Ashraf *et al.,* 1998). The higher concentration of Na^+ in root and shoot retarded the uptake of K^+ and Ca^{2+}, which has been confirmed by Gadallah (1996) and Asghari *et al*., (2001) who recorded that Na^+ severity reduced the uptake of other necessary nutrient elements like K^+ and Ca^{2+}. K^+ is involved in many important metabolic processes like maintaining the water status of plant and turgor pressure of the cell. K^+ also plays a key role in regulating stomatal aperture and is required for accumulation and translocation of carbohydrates. It is also co-factor of some enzymes (Nabil & Coudrest, 1995; Talbott & Zeiger, 1996).

The concentration of K^+ was decreased with the increase in the concentration of NaCl. At 2% NaCl treatment, although K^+ was less than control but provenance II accumulated maximum K^+ in root whereas it was least in provenance IV. Minimum concentration of K^+ was obtained at the highest salinity levels. Decreased uptake of K^+ under NaCl stress has been documented previously (Talbott & Zeiger, 1996). The uptake of K^+ may affect the metabolic activities essential for growth as observed during the present investigation for provenance II and III (Table 2). Present study confirmed that the provenances with lower K^+ content had poor growth while provenance II and III maintained higher K^+ and showed better performance. The results are also supported by Hussain *et al.*, (2002), Lacan & Durand, (1995) and Alhugdow *et al*., (1999).

The concentration of K^+ in root was less than that of shoot in all the provenances which indicated that K is mobile element and its maximum requirement is in leaves to control different metabolic activities. However, provenance II and III had relatively higher concentration of K^+ in root. Talbott & Zeiger, (1996) reported decrease in K^+ concentration due to high concentration of salt even in tolerant plants (Asghari *et al.,* 2001).

NaCl treatment significantly reduced Ca^{2+} both in shoot as well as in root in all the provenances of *Eucalyptus*. At 2% NaCl, there was no significant difference among the provenance for the accumulation of Ca^{2+} in shoot while, in case of root, provenance II and III showed maximum concentration of Ca^{2+}. The tolerant provenances maintained higher concentration of Ca^{2+} at 0.5 and 1% NaCl concentrations after the control as well as in saline conditions, as compared to the relatively tolerant one as also reported by Shabala *et al*., (2003) and Khan *et al*., (2000) in *Atriplex griffithii*. Similar results were reported by Bell *et al*., (1990); and Asghari *et al*., (2001) in wheat.

K/Na ratios decreased with the increase in NaCl concentration both in shoot and root in all the provenances. At low NaCl concentration (0.5%), the K/Na ratio was higher in all the provenances and it was highest in provenance I after the control which showed marked decrease with the increase in NaCl, but in shoot the extent of decrease in provenance II and III was less with the increase in NaCl. At the highest salinity level (2% NaCl concentration), the K/Na ratio in all the provenances was similar in case of shoot while in case of root, K/Na ratio was higher in provenance II and III. It seems that K/Na role in osmotic adjustment could be related with partitioning. Both these should effect on reclamation of salt. Similar results were also reported by Gorham & Wyn Jones (1993) in wheat, Alhugdow *et al*., (1999) in potato and Hussain *et al*., (2002) in barley.

The high K/Na ratio was taken as an index for salt tolerance as reported previously by Bohra *et al*., (1995). Jaenicke *et al*., (1996) in *Leucaena leucocephala*. The present

study revealed that provenance II has the ability to maintain higher K/Na ratio, and showed better growth and biomass production (Table 3). Hence, the K/Na ratio can be used as a selection criterion for the salt tolerant plants as also reported by Alhugdow *et al*., (1999). The K/Ca ratio was significantly enhanced in shoot with the application of NaCl. It was maximum at the highest salinity level (2% NaCl) which indicated that concentration of Ca^{2+} was reduced at higher level than that of K^+ due to salinity (Gorham & Bridges, 1995). Similar results were reported by Cramer *et al*., (1985) and Asghari *et al*., (2001).

It is evident from the studies that *Eucalyptus* provenances which have high K^+ and Ca^{2+} contents may suppress the toxic effects of Na^+ and hence could be recommended for cultivation on highly salt-affected soils.

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