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SALINITY TOLERANCE POTENTIAL OF TWO ACACIA SPECIES AT EARLY SEEDLING STAGE

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Soil salinity is a major environmental issue in arid and semiarid regions of the world. Acacia has very important role for salt affected barren lands due to its high salinity tolerance potential. The aim of the present study was to explore the genetic differences among *Acacia ampliceps and Acacia nilotica* regarding their response to salinity. Three-weeks old seedlings of both species were transplanted in half strength Hoagland nutrient solution having five salt levels (control,100, 200, 300 and 400 mM NaCl) with four replications in completely randomized design with factorial arrangement. After eight weeks of transplantation, the plants were harvested and data for shoot and root length and their fresh and dry weights were recorded. Na^+, K^+ and Cl^-concentration of both root and shoot was determined. All the growth parameters of both species declined significantly in response to salinity. *Acacia ampliceps* was more salt tolerant than *Acacia nilotica* with better growth owing to higher K^+: Na^+ ratio in plant tissues.

Keywords: Salinity, NaCl, K^+: Na^+ ratio, acacia species, ionic composition

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INTRODUCTION

Yield of many crops particularly for arid and semi-arid areas of the world is reduced due to salinity ((Majeed *et al*., 2010; Murtaza *et al*., 2011). About, 7% of the global land area and approximately 20% global cultivated land is affected by salinity (Zhu, 2001). About 6.67 m ha, approximately one third of the total cultivated area in Pakistan is having the problem of soil salinization (Khan, 1998).

The growth of most of the plants is badly affected due to salinity (Taiz and Zeiger, 2006) and if the level of salinity is too high the growing plants may be eliminated totally (Garg and Gupta, 1997). The provision of all the nutrients to plant is made through roots so the reaction of roots to salinity is very vital for understanding the whole plant response to salinity (Ahmed *et al*., 2012). Plant cells, tissues and organs display varied response to harsh environmental conditions at different levels of their growth (Munns, 1993). High cytoplasmic solute concentrations cannot be tolerated by most of the plants, so in order to carry on routine metabolic functions, plants have to dump higher concentrations of salts in their vacuoles or they have to restrict them in older tissues (Munns and Tester, 2008). Potassium ion is very vital for many cell functions like cell enlargement, osmoregulation and homeostasis (Schachtman *et al*., 1997). The plants with higher K^+: Na^+ ratio are considered better tolerant to salinity stress (Saqib *et al*., 2005). Despite decline in growth due to salinity, there is marked genotypic difference within plant species regarding their sensitivity to higher concentrations of salts (Saqib *et al*., 2006).

According to Saboora *et al*. (2006), it is expected that salinity will increase at a rate of about 10% per year around the globe. Grassland cover and ultimately the feed production for animals is reduced due to salinization in the areas of arid and semiarid climate (El-kharbotly *et al*., 2003). There is a great demand for such plant species which are naturally gifted with high salinity tolerance potential and at the same time can give reasonable economic yield from such barren lands.

Acacia species have the ability to survive in a varied range of territories and environments and are well acclimatized to the semi-arid and savannah type of climates and are tolerant to high pH and waterlogging (Benninson and Paterson, 1993). This genus is highly tolerant to salinity as well. Yokota (2003) compared five acacia species, viz *A. ampliceps, A. salicina, A. ligulata, A. holosericea* and *A. mangium* in solution culture for their salt tolerance and found that *A. ampliceps* was more tolerant and survived even at 428 mM NaCl where all the other species died. Marcar *et al*. (1991) found that some acacia species have the potential to grow up to 400 mM NaCl concentration. The objective of this study was to evaluate the salinity tolerance potential of two acacia species for their growth and ionic relationship, so that the most promising specie can be recommended for salt-affected lands.

MATERIALS AND METHODS

Seeds of two acacia species (*A. nilotica* and *A. ampliceps*) were collected from Forest Research Institute, Peshawar, Pakistan and Nuclear Institute for Agriculture and Biology

(NIAB), Faisalabad, Pakistan. The seeds were sown in iron trays having two inch layer of sand washed with distilled water. The sand was kept moistened with water and with nutrient solution after seedling emergence. After three weeks, the seedlings were transplanted in foam plugged holes in polystyrene sheets floating over nutrient solution in 25L iron tubs.

After one week of transplantation, different levels of salinity (100, 200, 300 and 400 mM NaCl) were developed by the addition of calculated amount of NaCl in two increments (one per day) in the salinity treatment tubs, whereas no salt was added in control. The pH of the solution was maintained at 5.5±1 with dilute NaOH or HCl and the solution was changed weekly during the period of study. After eight weeks, the plants were harvested and the data regarding shoot and root length and fresh weights were recorded. Oven dry weight of shoot and root was recorded after drying the samples at 75ºC for 48 hours. The shoot and root samples were ground in a grinding machine and digested with H_2SO_4 and H_2O_2 following the method of Wolf (1982) and were analyzed for ions. Na^+ and K^+ were determined by Sherwood- 410 Flame Photometer and Cl^- was determined with Sherwood- 926 chloride analyzer. Data were subjected to statistical analysis (Steel *et al*., 1997) and significance of differences among treatments and species was determined using LSD test.

RESULTS

***Shoot and root growth*:** The increasing levels of salinity resulted in reduction in shoot fresh and dry weights of both acacia species. The differences among species, treatments and their interaction were significant for both these parameters (Table 1). *A. ampliceps* survived even at 400 mM NaCl whereas *A. nilotica* could not survive at this salinity level (data not shown). In control, both species did not differ significantly; however, in the salt treatments they differed significantly from each other and this difference widened with increasing salt concentration in the growing medium. Salinity resulted in decline in the root growth as well. The main effects as well as their interaction were significant for both root fresh and dry weights. Reduction in fresh and dry weights of root was more in case of *A. nilotica* than *A. ampliceps* (Table 1). The length of both shoot and root also decreased due to salinity stress. Although treatments had significant effect; the species did not differ significantly from each other (Table 1). *A. nilotica* had higher root and shoot length up to 200 mM NaCl level. However, in 300 mM NaCl treatment, *A. ampliceps* had higher length of both root and shoot.

***Shoot and root ionic composition*:** Salinity significantly increased the shoot and root Na^+ concentration in both species. (Table 2). The individual as well as interactive

Table 1. Effect of salinity on growth parameters of acacia species

	Control	100 mM NaCl	200 mM NaCl	300 mM NaCl	Mean
A. Shoot fresh weight (g plant^{-1})					
A. ampliceps	3.91 a	3.40 b	2.52 d	1.25 f	2.77 A
A. nilotica	3.74 a	3.00 c	1.87 e	0.50 g	2.28 B
Mean	3.83 A	3.20 B	2.19 C	0.88 D	
B. Shoot dry weight (g plant^{-1})					
A. ampliceps	0.74 a	0.63 b	0.49 d	0.24 f	0.53 A
A. nilotica	0.71a	0.55c	0.36 e	0.11 g	0.43 B
Mean	0.73 A	0.59 B	0.43 C	0.18 D	
C. Root fresh weight (g plant^{-1})					
A. ampliceps	1.78 a	1.60 b	1.20 d	0.65f	1.31 A
A. nilotica	1.74 a	1.44 c	0.95 e	0.33 g	1.12 B
Mean	1.76 A	1.52 B	1.08 C	0.49 D	
D. Root dry weight (g plant^{-1})					
A. ampliceps	0.31 a	0.28 b	0.22 d	0.12f	0.23 A
A. nilotica	0.30 a	0.24 c	0.17e	0.06 g	0.19 B
Mean	0.31 A	0.26 B	0.20 C	0.09 D	
E. Shoot length (cm)					
A. ampliceps	30.0 b	26.5 c	18.25 d	9.50 e	21.06 A
A. nilotica	34.0 a	28.5 bc	19.0 d	5.60 f	21.78 A
Mean	32.0 A	27.5 B	18.63 C	7.55 D	
F. Root length (cm)					
A. ampliceps	24.0 b	21.5 c	16.0 d	8.10 e	17.4 A
A. nilotica	27.0 a	23.1 bc	16.5 d	4.70 f	17.83 A
Mean	25.5 A	22.3 B	16.3 C	6.40 D	

Means of a parameter sharing the same small letters are statistically similar at P ≤ 0.05.

Table 2. Effect of salinity on ionic composition of acacia species

	Control	**100 mM NaCl**	**200 mM NaCl**	**300 mM NaCl**	**Mean**
A. Shoot Na^+ concentration (mmol g^{-1} dw)					
A. ampliceps	0.15 f	0.51 e	0.91 d	1.72 b	0.82 B
A. nilotica	0.15 f	0.76 d	1.30 c	2.30 a	1.13 A
Mean	0.15 D	0.64 C	1.11 B	2.01 A	
B. Root Na^+ concentration (mmol g^{-1} dw)					
A. ampliceps	0.19 g	0.65 f	1.30 d	2.10 b	1.06 B
A. nilotica	0.19 g	0.90 e	1.91 c	2.86 a	1.47 A
Mean	0.19 D	0.78 C	1.61 B	2.48 A	
C. Shoot Cl^- concentration (mmol g^{-1} dw)					
A. ampliceps	0.19 g	0.60 f	1.01d	2.10 b	0.98 B
A. nilotica	0.18 g	0.76 e	1.41 c	2.62 a	1.24 A
Mean	0.19 D	0.68 C	1.21 B	2.36 A	
D. Root Cl^- concentration (mmol g^{-1} dw)					
A. ampliceps	0.24 g	0.65f	1.34 d	2.47 b	1.18 B
A. nilotica	0.27 g	0.90 e	1.86 c	3.21 a	1.56 A
Mean	0.26 D	0.76 C	1.6 B	2.84 A	
E. Shoot K^+ concentration (mmol g^{-1} dw)					
A. ampliceps	1.22 a	0.95 b	0.70 d	0.50 e	0.85 A
A. nilotica	1.20 a	0.82 c	0.55 e	0.29 f	0.71 B
Mean	1.21 A	0.89 B	0.60 C	0.42 D	
F. Root K^+ concentration (mmol g^{-1} dw)					
A. ampliceps	0.76 a	0.65 b	0.50 d	0.35 e	0.57 A
A. nilotica	0.75 a	0.58 c	0.38 e	0.19 f	0.48 B
Mean	0.76 A	0.62 B	0.44 C	0.27 D	

Means of a parameter sharing the same small letters are statistically similar at $P \leq 0.05$.

effects of treatment and species were significant. In control, both species had same concentration of Na^+. In saline treatments, *A. ampliceps* accumulated significantly lower Na^+ as compared to *A. nilotica* in both shoot and root. The difference between both species was maximum at 300 mM NaCl. In this treatment, maximum Na^+ concentration (2.3 mmol g^{-1}dw) was found in the shoot of *A. nilotica.* At the same salinity level shoot Na^+ concentration was 1.72 mmol g^{-1}dw in *A. ampliceps*. The root Na^+ concentration in 300 mM NaCl treatment was 2.86 and 2.1 mmol g^{-1}dw for *A. nilotica* and *A. ampliceps*, respectively. Salinity significantly increased the shoot and root Cl^- concentration in both species (Table 2). The individual as well as interactive effects of treatment and species were found significant. In all saline treatments, *A. ampliceps* accumulated significantly lower Cl^- as compared to *A. nilotica* in both shoot and root. At 300 mM NaCl, maximum Cl^- concentration in the shoot of *A. nilotica* was 2.62 mmol g^{-1}dw. Shoot Cl^- concentration was 2.1 mmol g^{-1}dw in *A. ampliceps*. At this salinity level the root Cl^- concentration was 3.21 and 2.47 mmol g^{-1}dw for *A. nilotica* and *A. ampliceps* respectively. The concentration of K^+ as against of Na^+ decreased significantly at each increasing level of salinization (Table 2). The individual effects of treatments and species as well as their interaction were found significant. In control, K^+ concentration was the maximum whereas it was the minimum in 300 mM NaCl. The comparison of species at each salinity level showed that *A. ampliceps accumulated* more K^+ in both organs as compared to *A. nilotica.* At 300 mM NaCl, shoot K^+ concentration was 0.55 mmol g^{-1}dw in *A. ampliceps* whereas in case of *A. nilotica* it was 0.29 mmol g^{-1}dw. At this salinity level the root K^+ concentration was 0.35 and 0.19 mmol g^{-1}dw for *A .ampliceps* and *A. nilotica*, respectively.

The K^+: Na^+ ratio was maximum in control and decreased with increasing salinity levels. This ratio was minimum in 300 mM NaCl treatment. *A. ampliceps* had higher K^+: Na^+ ratio than *A. nilotica* for both root and shoot under saline conditions (Fig.1&2).There was a strong negative correlation of Na^+ and Cl^- concentration with shoot dry weight of both acacia species (Fig. 3&4). On the other hand a strong positive correlation was found between shoot K^+ concentration and shoot dry weight of both species (Fig. 5).

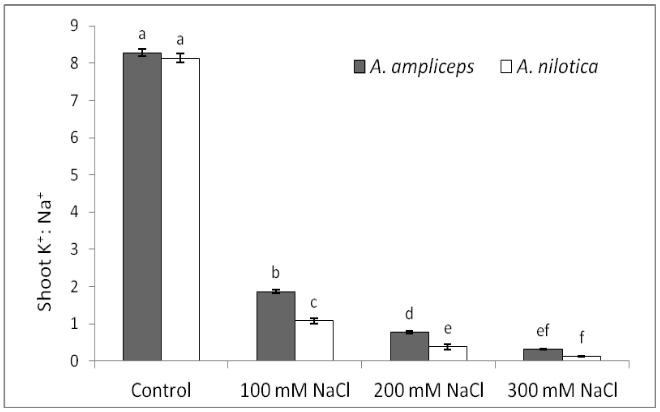

Figure 1. Effect of salinity on shoot K^+: Na^+ ratio of acacia species. For both species vertical bars having a common letter are not significantly different at $P < 0.05$.

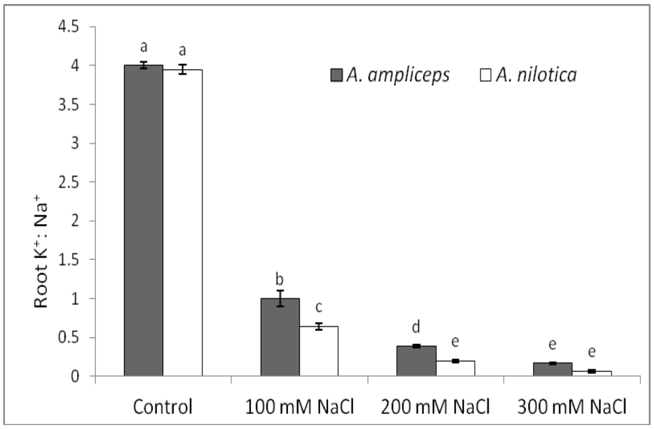

Figure 2. Effect of salinity on root K^+: Na^+ ratio of acacia species. For both species vertical bars having a common letter is not significantly different at $P < 0.05$.

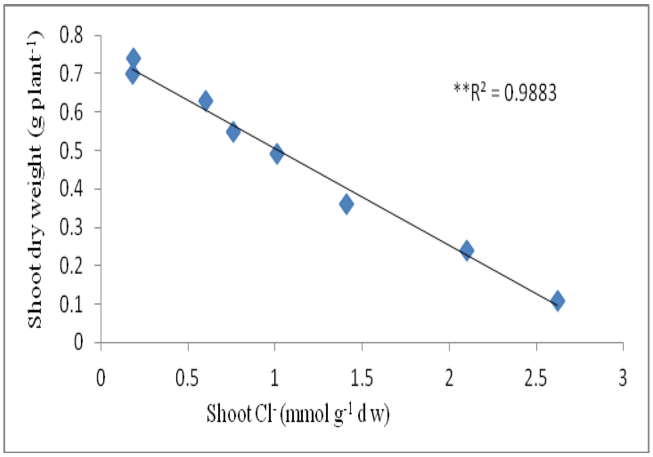

Figure 3. Correlation between shoot dry weight and Cl^- concentration of acacia species

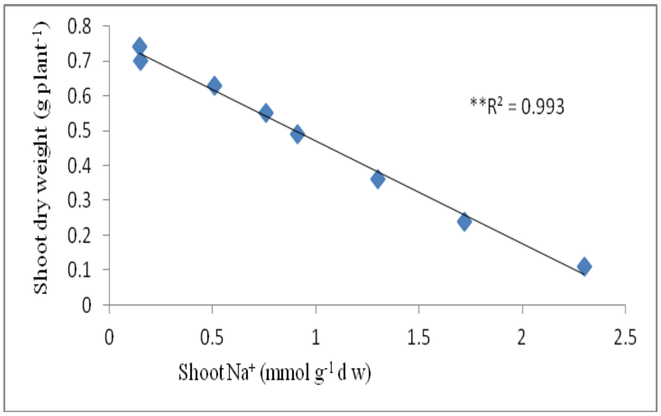

Figure 4. Correlation between shoot dry weight and Na^+ concentration of acacia species

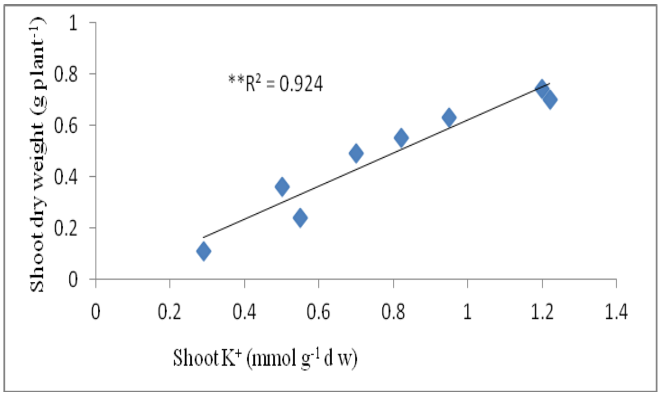

Figure 5. Correlation between shoot dry weight and K^+ concentration of acacia species

DISCUSSION

This study indicated high salinity tolerance potential of the two acacia species, i.e. *A. amplicep* and *A .nilotica*. However, there was a distinct genetic difference between the species regarding salinity tolerance. *A. ampliceps* survived even at 400 mM NaCl whereas this salinity level proved to be fatal for *A. nilotica*. Such genetic variability in acacia was also observed by Marcar *et al*. (1991) who found that in a sand culture experiment, *A. ampliceps* and *A. auriculiformis* survived at 400 mM NaCl whereas *A. mangium* died at this salinity.

Shoot fresh and dry weights of both acacia species declined with increasing levels of salinization and maximum reduction was noticed at 300 mM NaCl. Beyond this salinity level, *A. nilotica* could not survive, however the seedlings of *A. ampliceps* survived. On relative basis, shoot dry weight was decreased up to 68 and 85% as compared to control in *A. ampliceps* and *A. nilotica* respectively. Salinity induced reduction in shoot growth in acacia was also reported by Farooq *et al*. (2010) and Yokota (2003). Reduction in the growth of plants due to salinity is mainly attributed to three principle factors i.e. osmotic effects, ion toxicity and deficiency of necessary nutrients (Munns and Tester, 2008). These factors are operative at both cellular and whole plant level and manipulate all the metabolic activities of plants (Garg and Gupta, 1997). Ion toxicity and reduced osmotic potential of rooting medium are the main causes of reduced fresh and dry weights of shoot (Munns *et al*., 1995).

Like shoot growth, the growth of root also decreased to a great extent due to salt treatments as compared to control. On relative basis, reduction in root dry weight as compared to control was 80 and 61% in *A. nilotica* and *A. ampliceps*, respectively. The length of both root and shoot was more in *A. nilotica* than *A. ampliceps* in control as well as in 100 and 200 mM NaCl levels. However, in these treatments the weight of both root and shoot was more in *A. ampliceps* than *A. nilotica*. The reason is that the leaves of *A. ampliceps* are much fleshy and heavier than those of *A. nilotica* and the roots of *A. ampliceps* have more lateral growth as compared to *A. nilotica*. In 300 mM NaCl level, there was severe reduction in length of both root and shoot; in this treatment *A. ampliceps* produced more shoot and root length than *A. nilotica*. Reduction in root growth due to salinity was also found by Hardikar and Pandey (2008) and Mahmood *et al*. (2010).

At higher levels of salinity both root formation (Kramer, 1983) and their elongation (Garg and Gupta, 1997) is reduced greatly. So both water and salt stress reduce the plant growth in an additive manner. The physical growth parameters like fresh and dry weights of both root and shoot are usually related to salinity tolerance potential of all plants at their early stages of growth so these are the tools of selection criteria for salinity tolerance (Larcher, 1995). Plants also have phytoremediation potential (Zulfiqar *et al.*, 2012).

The ionic composition revealed that shoot and root Na^+ and Cl^- concentration of both species increased significantly in response to increasing salinity levels with more accumulation in *A. nilotica* than *A. ampliceps*. This higher accumulation of Na^+ in cells corresponds to the lower growth and dry weights of *A. nilotica*. These results are also supported by the findings of Marcar *et al*. (1991). The buildup of toxic ions in plant tissues is thought to be the major factor of decline in growth under salinity stress (Muscolo *et al*., 2003). There was a strong negative correlation of Na^+ and Cl^- concentration with shoot dry weight (Fig. 3&4). On the other hand, a strong positive correlation was found in case of shoot K^+ concentration and shoot dry weight (Fig. 5). Ahmed *et al*. (2012) found an inverse correlation between the shoot growth and Na^+ concentration in maize genotypes and Arshad *et al.* (2012) and Saqib *et al.* (2012) found similar relationship in wehat. Higher concentration of Na^+ in tissues causes nutrient imbalance, osmotic effects and specific ion toxicity (Arzani, 2008).

K^+ has a key role in salt tolerance where uptake of K^+ is decreased by Na^+ (Fox and Guerinot, 1998). In our study, the reduction of K^+ concentration was found in both root and shoot which indicated that Na^+ repressed the uptake of K^+. The replacement of K^+ for Na^+ in the stele of the roots or in the vascular bundles in stems is thought as a mechanism for controlling transportation of salts from roots to shoots (Hardikar and Pandey, 2008). The higher concentration of Na^+ in shoots of *A. nilotica* indicated that this mechanism was poorly operative in this species so it had low K^+ concentration as compared to *A. ampliceps*. Decreased K^+ uptake in response to salinity was also observed by (Ahmed *et al*., 2012; Marcar *et al*., 1991). The plants with higher K^+: Na^+ ratio are considered more tolerant to salinity stress (Saqib *et al*., 2005). Similar was the case in our results where *A. ampliceps* owing to higher K^+: Na^+ ratio showed better salt tolerance. The higher Na^+, Cl^- and lower K^+: Na^+ resulted in the reduction of root and shoot growth of *A. nilotica* more as compared to *A. ampliceps*.

***Conclusion*:** Although salinity had a detrimental effect on all the growth parameters of the both acacia species, there was a distinct genetic difference between the tested species. On the basis of better ionic homeostasis *A. ampliceps* had more growth and survival at higher salinity levels than *A. nilotica*. Therefore, it can be successfully grown on highly salt-affected lands in order to get economic yield from otherwise barren lands.

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