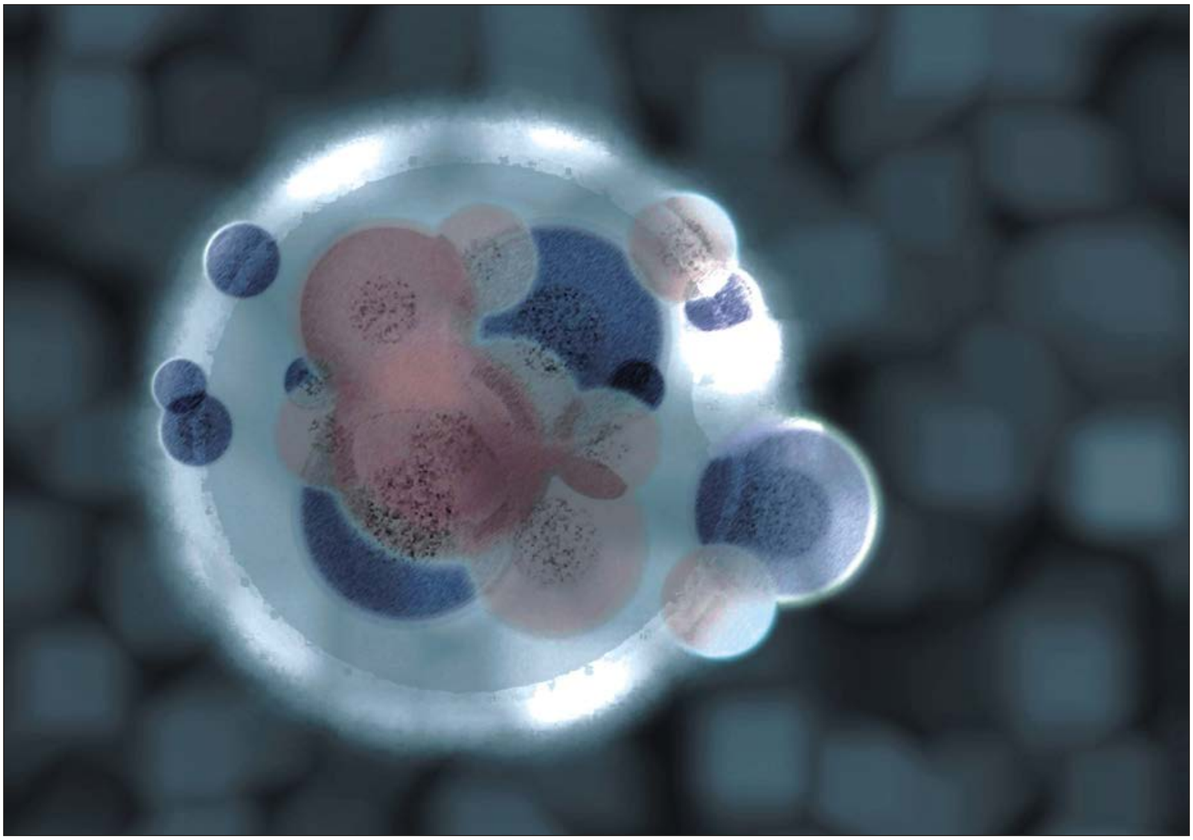

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Effect of Different Levels of Salinity Stress on Growth and Morphological Characteristic of Two Legumes

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Abstract: The objective of this study was to investigate the sensitivity of morphological and physiological responses of burr medic (*Medicago polymorpha* L. cv. Scimitar) and balansa clover (*Trifolium michelianum* L. cv. Frontier) to low levels of salinity. Morphological responses determined included leaf width, leaf length, plant height and dry matter yield. The results indicated that sodium (Na^+) and potassium (K^+) content in both leaves and stem tissues increased with increased salt levels. Sodium contents in leaves under 80 mM salt levels were 3-fold and 2-fold than for the control for balansa clover and burr medic, respectively. Leaf Na^+/K^+ ratio remained relatively constant in both balansa clover (0.3-0.4) and burr medic (0.4-0.5). Stem Na^+/K^+ ratios ranged between 0.3-0.7 in balansa clover and between 0.4-1.0 for burr medic. These results indicate that burr medic is much more high-yielding and seem to tolerate the levels of salinity just as well as balansa clover.

Key words: Salt-tolerant, clover, morphological parameters

INTRODUCTION

Dry land salinity is a global problem that costs millions of dollars each year in lost yields and damaged infrastructure. Recent studies have estimated that in Australia more than 4×10^6 ha of land has been affected by secondary salinity (Bell, 1999) and most of the dryland salt-affected areas are in Western Australia. More recently the ANZECC Task Force (2001) indicated that 5.7 m ha are currently affected or at risk from dry land salinity.

Plant-based solutions involving perennial legumes provide an economically viable option for management of dry land salinity. A recent report has revealed a list of potential fodder plants, including perennial legumes such as burr medic (*Medicago polymorpha* cv. Scimitar) and balansa clover (*Trifolium michelianum* L. cv. Frontier), that provide the best option for revegetation of mild to moderately salt-affected lands (Rogers *et al*., 2005). However, an understanding of the range of salinity that various legumes can tolerate is central to successful revegetation of saline lands using agronomic crops.

The mechanisms of salt tolerance take place at three levels of organization, viz. whole plant, cellular and molecular. At the whole pant level salt tolerance depends on the ability of the plant to regulate the transport of salt at various tissues within the plant (Munns and James, 2003). Extensive reviews exist in the literature on salinity tolerance (Greenway and Munns, 1980; Jacoby, 1999). It is now commonly agreed that plant species use more than one strategy for avoidance of or tolerance to salt stress. Defense against excess entry of sodium into the plant firstly occurs at the root cell plasma lemma, which is known to have a low permeability for Na^+ (Dear *et al*., 1998). In contrast, root cells exhibit preferential uptake of K^+ which can be accumulated against a concentration gradient. Salt tolerance is largely a question of maintaining a balance between allowing sufficient salt to enter the shoot for osmotic adjustment and preventing the accumulation of toxic levels of salt within the plant (Gorham, 1996).

Research indicates that the most tolerant species actually have high internal salt concentrations such that plant survival depends strongly on the ability to restrict accumulation of salt (Gorham, 1996; Kozlowski, 1997). In halophytes, the salt sequestration occurs in the vacuoles and thus reduces the salt concentration to which the cytoplasm and chloroplasts are exposed. Another form of osmotic adjustment may also occur through synthesis in the cytoplasm of compatible organic solutes such as glycine betaine, proline, polyols and sugars. The presence of high concentrations of organic compounds in the cytoplasm counterbalances the high salt concentrations in the vacuoles (Kozlowski, 1997; Jacoby, 1999). Salt tolerance varies considerably with many environmental and plant factors (species,

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variety, stage of growth) (Kozlowski, 1997). Some researchers have indicated that plants that are more able to tolerate moderately saline environments have great ability to exclude Na^+ from the shoot or at least leaf blades and at the same time maintain high levels of K^+ (Tester and Davenport, 2003). Some non-halophytes have been found to control the transport of salt to the leaves reasonably well, especially in low to moderately saline environments. For example, total fruit yield in bell-pepper hybrids was significantly reduced at salinities higher than 10 mM NaCl, the reduction being 95% at 150 mM NaCl (Chartzoulakis and Klapaki, 2000).

The objective of this study was to investigate the relative sensitivity of morphological traits of two legumes, burr medic (*Medicago polymorpha* L. cv. Scimitar) and balansa clover (*Trifolium michelianum* cv. Frontier) to low and moderate levels of salinity stress. Specifically, the goal was to elucidate better the relationship between salinity and growth.

MATERIALS AND METHODS

Experimental design and growth conditions: The experiment was conducted in the University of Western Australia during January-March 2005. The average day and night temperatures were 25±3 and 16±°C, respectively. The study was arranged in the glasshouse as a randomized complete block design with two legume species, four levels of salinity and three replicates. The two perennial legumes, balansa clover and burr medic were used for the study. The four salinity levels were control (non-saline, 0 mM NaCl), mild salinity (20 mM NaCl), moderate salinity (40 mM NaCl) and high salinity (80 mM NaCl). A sandy soil from Lancelin with $EC_{1:5}$ of 0.04 dS m^{-1} and $pH_{1:5}$ of 6.1 was used for the experiment. Gravel was placed at the bottom of each pot to promote drainage.

Fifteen seeds were planted at depths of 6-7 mm for both balansa clover and burr medic in each pot. Seeds of both species were inoculated with rhizobia (*Rhizobium meliloti* for balansa clover) before planting. After 3 weeks, the plants were thinned to five plants per pot. For balansa clover this was equivalent to a seeding rate of 4 kg ha^{-1}, while for burr medic this was equivalent to a seeding rate of 10 kg ha^{-1}. To avoid salt the application of NaCl was conducted in increments of 20 mM per week starting at three weeks after germination. The study was conducted under normal light conditions.

Nutrient solution containing phosphorus, potassium, sulphur and several micronutrients was added before seeding. Aliquots of 12 mL each containing nutrient solutions of KH_2PO_4 (22.4 g L^{-1} for K and P supply), $CaCl_2$ (21.3 g L^{-1} for Ca supply) and $MgSO_4.7H_2O$ (5.96 g L^{-1} for S and Mg supply) were applied in each pot of with 4 kg^{-1} of soil.

Growth and morphological traits: Plant height was determined using a ruler at initial (before salt addition) and final stages (before harvest). Width and length of the third trifoliolate were determined at initial and final stages using a Vernier calliper. At the end of the study, one plant was randomly selected for collecting xylem sap and the remaining four plants were harvested, had their fresh weight determined before oven-drying at 70°C.

Sodium, potassium and calcium concentrations in shoots: For acid digestion, finely-ground oven-dried leaf and stem sub-samples of 0.5 g each were placed in 50 mL flasks and 10 mL of concentrated nitric acid added. The mixture was first heated on fry pans to 90°C for 30 min, before increasing the temperature to 140°C in order to boil off excess nitric acid. When approximately 1 mL of liquid was left the flasks were cooled off before transferring the extracts into 23 mL vials. De-ionised water was added to make up to the 23 mL volume of the primary extract. An aliquot of this primary extract was diluted for determination of sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) concentrations using the atomic absorption spectrophotometer (AAS, Perkin Elmer Analyst 300). To minimize ion interferences a solution of lanthanum oxide was added to each diluted extract to give a lanthanum content of 0.1%. The Na^+/K^+ and Na^+/Ca^+ ratios were calculated to determine the extent of ion selectivity in leaves and stems of balansa clover and burr medic under different salinity levels.

Xylem sap collection, osmolality and ion composition: Xylem sap was collected using the pressure chamber method (Ulrich, 1998). The procedure involved cutting one plant from each pot one plant was cut at the base. About 30 mm around the cut end was stripped off the bark using surgical blades. The whole plant was carefully inserted into the pressure chamber with only the cut end protruding through the chamber gasket. Pressure was applied slowly until the liquid started coming out through the cut end. The first 30-40 μL of sap were discarded to avoid any contamination. Up to 300 μL of xylem sap was collected from each plant, placed into vials and immediately frozen using liquid nitrogen. Before measurements of xylem osmolality, samples were unfrozen for about 15-20 min. Aliquots of 10 μL of xylem sap were inserted into a specialized medical vial for measurement of osmolality using the Fiske 110 freezing point osmometer. Xylem sap Na^+, K^+ and Ca^{2+} analysis were conducted using the atomic absorption spectrophotometer (Perkin Elmer Analyst 300) after diluting a 100 μL aliquot. The determined ion concentrations were used to calculate Na^+/K^+ and Na^+/Ca^{2+} ratios of the xylem saps of both balansa clover and burr medic under four salt treatments.

Data analysis: Statistical analyses of all the morphological and physiological parameters were conducted using the SAS statistical package (SAS Institute, 2000).

RESULTS

Growth and morphological traits: The results in Table 1 indicate the initial (before salt treatments) and final (after salt treatments) plant heights, leaf width and leaf lengths, as well shoot biomass yield at the end of the study. The species and salt interaction had a significant effect ($p = 0.041$) on final plant height (adjusted for initial plant height). For the balansa clover the 20 and 40 mM treatments had lower final plant height than the control treatment and the 80 mM treatment had unexpectedly similar final plant height to that for the control treatment. However, for the burr medic there were non-significant differences in among the salt treatments. The final leaf width of the third trifoliolate did not significantly differ among salt treatments or between species. Similarly the final leaf length of the third trifoliolate did not differ significantly among salt treatments or between species. However, the shoot biomass yield was significantly affected by the species×salt interaction ($p = 0.044$). For balansa clover, the shoot biomass yield was greatest for the control treatment and lowest for the 20 mM treatment. The shoot biomass yields for 40 and 80 mM treatments did not differ from the control treatment. For burr medic, the shoot biomass yield did not differ among salt treatments.

Xylem sap osmolality, ion concentrations and ion ratios: Figure 1 indicates the xylem sap osmolality of balansa clover and burr medic at different levels of salinity. Statistical analyses indicated that there were no

Table 1: Initial height and final height (adjusted) of Balansa clover and Burr medic under different levels of salinity

Species	Salt level (mM)	Plant height (cm)	Leaf width (cm)	Leaf length (cm)	Shoot biomass (g pot^{-1})
23 March 2005-Initial height before salt addition					
Bal	0	11.87	1.28	1.71	
	20	10.50	1.08	1.43	
	40	12.29	1.14	1.52	
	80	12.21	1.29	1.69	
Bur	0	15.80	1.40	1.68	
	20	16.73	1.39	1.64	
	40	17.93	1.31	1.63	
	80	15.71	1.40	1.69	
22 April 2005-Final height (adjusted for initial height)					
Bal	0	24.26ab	1.38a	1.98a	8.5a
	20	21.33b	1.29a	1.76a	4.6b
	40	21.87b	1.29a	1.85a	6.3ab
	80	27.41a	1.27a	1.78a	7.0ab
Bur	0	31.30a	1.40a	1.78a	11.1a
	20	34.13a	1.31a	1.68a	13.2a
	40	32.43a	1.42a	1.80a	12.8a
	80	33.08a	1.36a	1.73a	12.1a

Within each column and for each species means followed by the similar letters are not significantly different at 5% significance level

significant differences in the xylem sap osmolality among salt treatments and also among plant species ($p = 0.15$). Averaged across replicates the osmolality for balansa

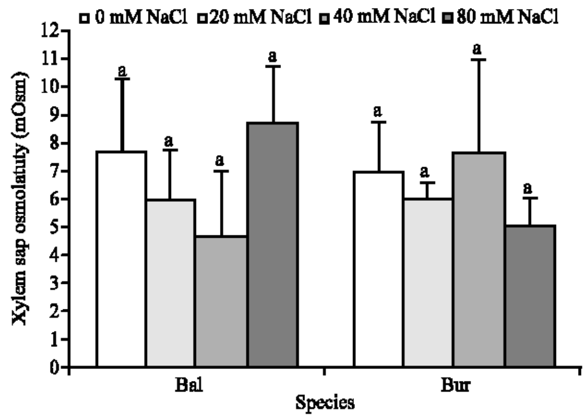

Fig. 1: Osmolality (mOsm) of xylem sap from balansa clover and burr medic after application of different levels of salts. Within each column and for each species means followed by the similar letters are not significantly different at 5% significance level

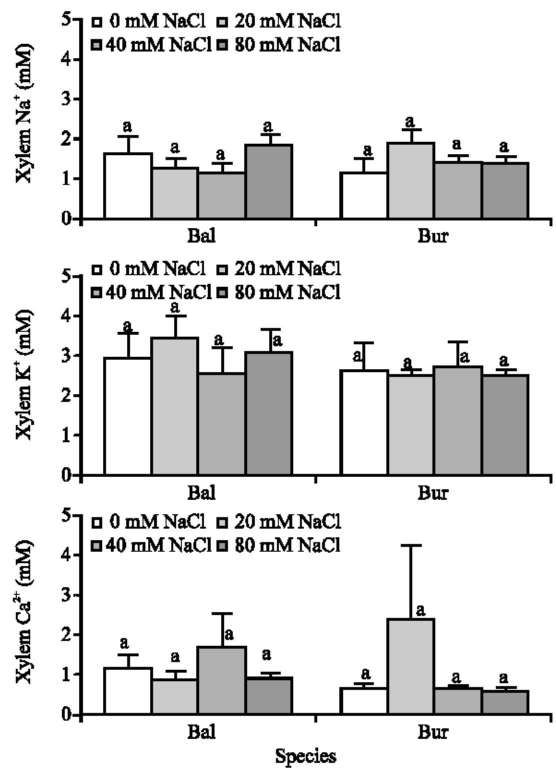

Fig. 2: Xylem sap Na^+, K^+ and Ca^{2+} concentrations (mM) in balansa clover and burr medic under four salt levels. Within each column and for each species means followed by the similar letters are not significantly different at 5% significance level

clover ranged from 4.7 to 8.7 mOsm, whereas that for burr medic ranged from 5 to 7.7 mOsm, indicating non-significant differences among salt treatments.

Ion analysis of the xylem sap indicated that the average Na^+ concentration in the xylem ranged from 1.25 to 1.88 mM for balansa clover and from 1.14 to 1.87 mM for burr medic (Fig. 2). The results of statistical analyses indicated non-significant differences between species and among salt treatments. Similarly, xylem K^+ and Ca^{2+} concentrations, as well as xylem Na^+/K^+ and Na^+/Ca^{2+} ratios did not significantly differ between species or among salt treatments (Fig. 2). For balansa clover, the Na^+/K^+ ratio of xylem sap ranged between 0.20-0.37 whereas, that for burr medic was between 0.27-0.43. However, the xylem Na^+/Ca^{2+} ratio was greater and ranged between 0.74-1.21 for balansa clover and was between 0.97-1.44 for burr medic.

Sodium, potassium and calcium concentrations in shoots: Sodium (Na^+) content in leaves was significantly affected ($p<0.0001$) by the salt levels applied, but there was no significant difference between burr medic and balansa clover. The salt levels in the leaf tissues for the 20, 40 and 80 mM NaCl treatments were 1.4-fold, 1.9-fold and 2.5-fold greater than that for the control treatment respectively. For balansa clover average leaf Na^+ ranged between 3.1-9.3 mg g^{-1} DW, with the lowest leaf Na^+ in the control treatment and the highest leaf Na^+ in the 80 mM treatment (Fig. 3). However, for burr medic the leaf Na^+ contents were between 3.4-6.9 mg g^{-1} DW and the lowest and the

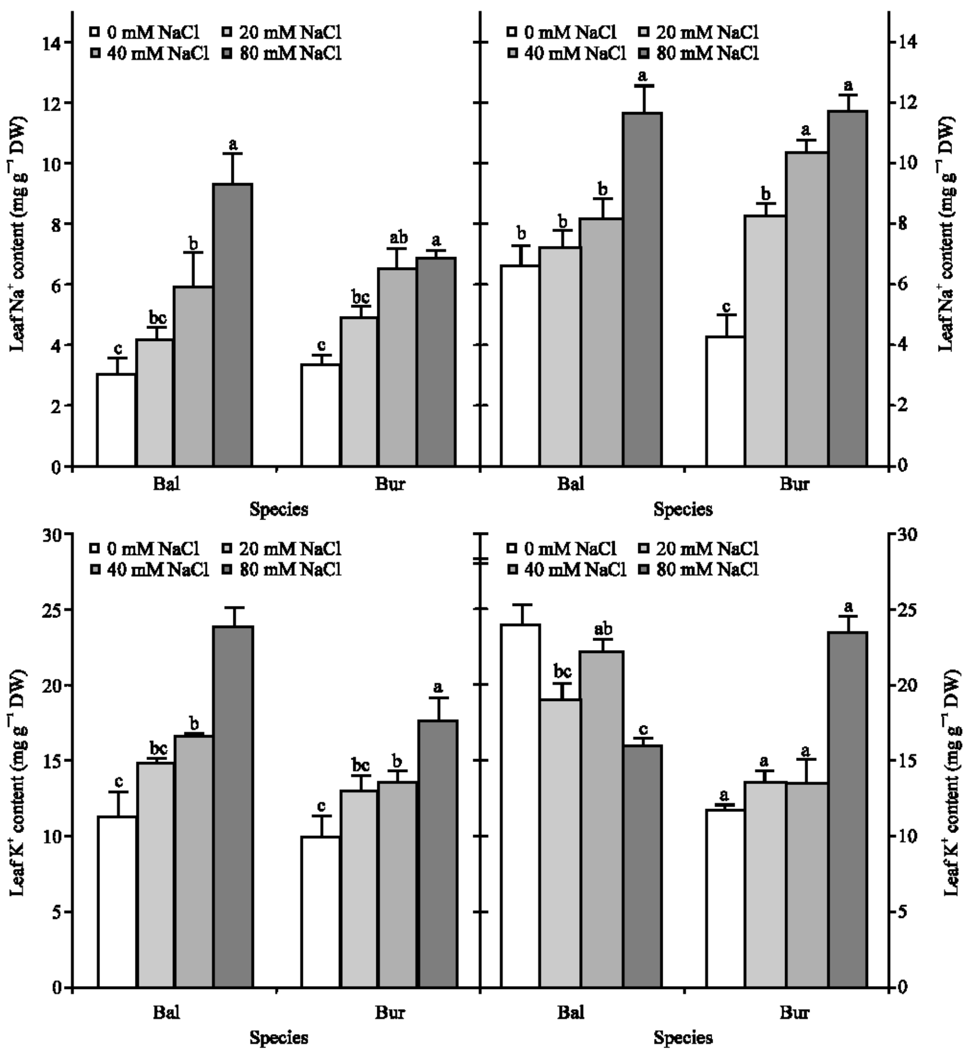

Fig. 3: Leaf and stem Na^+ and K^+ contents (mg g^{-1} dry weight) for balansa clover and burr medic under different salt levels. Within each column and for each species means followed by the similar letters are not significantly different at 5% significance level

highest leaf Na^+ values were observed in the control and 80 mM NaCl treatments, respectively. Stem Na^+ levels were generally slightly higher than leaf Na^+ for both balansa clover and burr medic (Fig. 2). The stem Na^+ range for balansa clover was 6.6-11.7 mg g^{-1} DW, whereas for burr medic the stem Na^+ ranged between 4.2-11.8 mg g^{-1} DW. The ratio of stem Na^+ to leaf Na^+ indicated a significant species and salt level interaction ($p = 0.046$) effect. The stem Na^+ to leaf Na^+ ratio for balansa was significantly higher (2.2) for the control than for the other salt treatments and ranged from 1.3 to 2.2. For burr medic the stem Na^+ to leaf Na^+ ratio did not significantly differ among salt levels and this ratio ranged from 1.4 to 1.7.

Leaf K^+ contents significantly differed between species ($p = 0.003$) and among salt levels ($p<0.0001$). In both balansa clover and burr medic the leaf K^+ in the 80 mM NaCl treatment was significantly different from that for the other salt treatments and the leaf K^+ in the 20 and 40 mM treatments were similar. However, leaf K^+ for the control was significantly lower than that in the 40 and 80 mM NaCl treatments (Fig. 3). For balansa clover, the average leaf K^+ was 16.7 mg g^{-1} DW and the range among salt levels was between 11.3-23.9 mg g^{-1} DW. However, for burr medic the average leaf K^+ was significantly lower (13.5 mg g^{-1} DW) and leaf K^+ for the different salt treatments ranged from 9.9 to 17.6 mg g^{-1} DW. With respect to stem K^+ an interactive effect existed between species and salt levels ($p = 0.01$). For balansa clover the stem K^+ content was the highest for the control, intermediate for the 20 and 40 mM NaCl treatments and

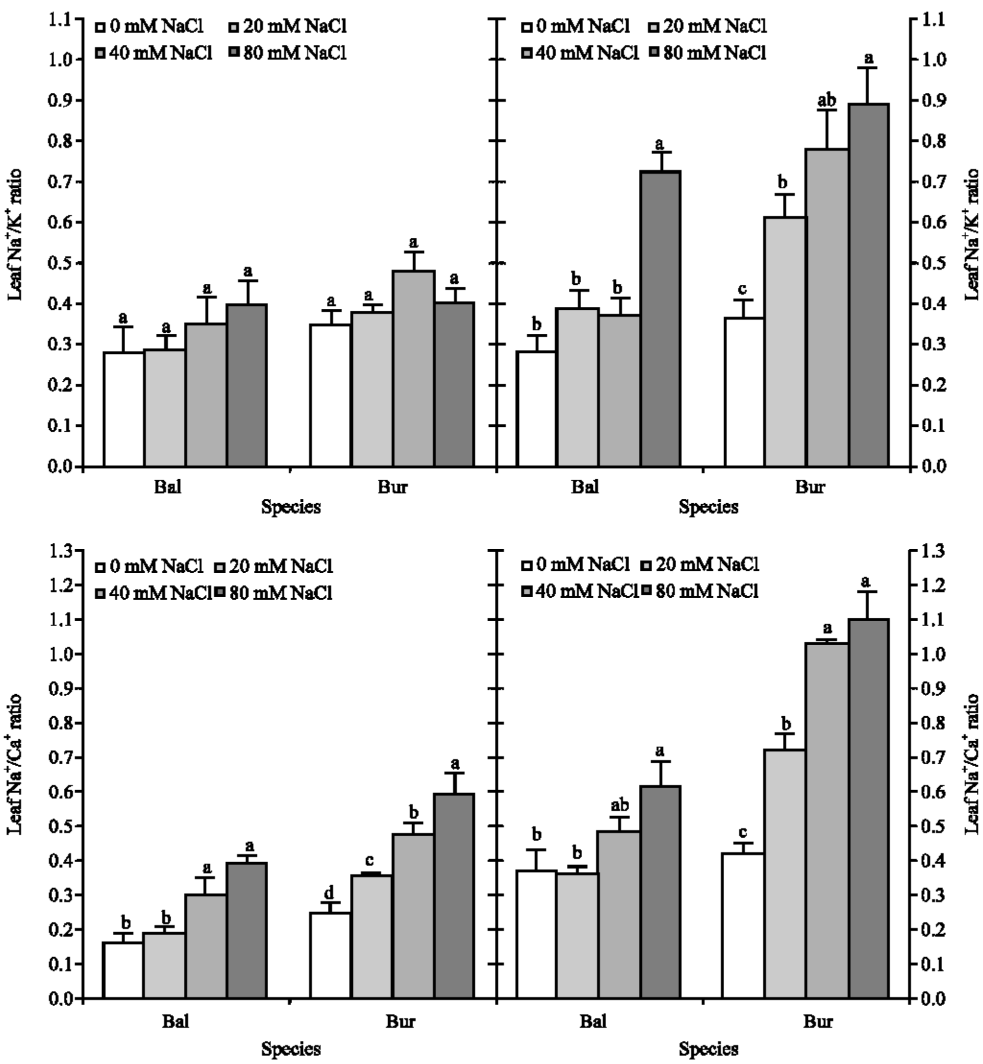

Fig. 4: Na^+/K^+ and Na^+/Ca^{2+} ratios of leaves and stems of balansa clover and burr medic under different salt levels. Within each column and for each species means followed by the similar letters are not significantly different at 5% significance level

lowest for the 80 mM NaCl treatment. However, for the burr medic the stem K^+ did not differ among salt treatments. The average stem K^+ content for balansa clover was 20.3 mg g^{-1} DW whereas that for burr medic was 13.2 mg g^{-1} DW. For balansa clover the range of stem K^+ content among salt levels was from 16.2 to 24.0 mg g^{-1} DW, whereas for burr medic the range was 11.5-13.6 mg g^{-1} DW.

Leaf Ca^{2+} was affected by interaction between species and salt levels (p = 0.01). For balansa clover leaf Ca^{2+} was lowest in the control treatment (19.3 mg g^{-1} DW) and highest for the 80 mM NaCl treatment (23.8 mg g^{-1} DW). However, for the burr medic the leaf Ca^{2+} did not significantly differ among salt levels and ranged from 11.9 to 14.1 mg g^{-1} DW. Stem Ca^{2+} was significantly different among species and not salt levels. The average stem Ca^{2+} for balansa clover was 18.8 mg g^{-1} DW whereas that for burr medic was 10.8 mg g^{-1} DW.

Figure 4 shows the leaf Na^+/K^+, stem Na^+/K^+, leaf Na^+/Ca^{2+} and stem Na^+/Ca^{2+} ratios calculated to determine ion selectivity between the two species and among the four salt levels. The leaf Na^+/K^+ ratio was non-significantly different among salt levels but differed significantly (p = 0.042) between species, with the leaf Na^+/K^+ ratio for burr medic (0.41) being higher than that for balansa clover (0.33). Stem Na^+/K^+ ratio significantly differed between species (p = 0.0001) and among salt levels (p<0.0001). For balansa, stem Na^+/K^+ ratio for the control treatment was lowest (0.28) whereas stem Na^+/K^+ for the 80 mM NaCl treatment was highest (0.73). Stem Na^+/K^+ ratio for burr medic followed a similar pattern, with the control treatment having the lowest ratio (0.36) and the 80 mM NaCl treatment having the highest stem Na^+/K^+ (0.73).

Leaf Na^+/Ca^{2+} ratio significantly differed between species (p<0.0001) and among salt levels (p<0.0001).The general range of the leaf Na^+/Ca^{2+} ratios was similar to that for the leaf Na^+/K^+ ratios. The leaf Na^+/Ca^{2+} for balansa clover was lowest in the control treatment (0.16) and highest in the 80 mM NaCl treatment (0.39). For burr medic, the leaf Na^+/Ca^{2+} ratio was also lowest in the control treatment (0.24) and highest in the 80 mM NaCl treatment (0.59). An interaction effect between species and salt levels was observed for stem Na^+/Ca^{2+} ratio. For balansa clover, the stem Na^+/Ca^{2+} ratio of 0.61 under the 80 mM NaCl treatment was much greater than that for other salt treatments. However, for burr medic stem Na^+/Ca^{2+} ratio of 0.42 was significantly lower than that for other salt treatments and stem Na^+/Ca^{2+} ratio for the 80 mM was greater than unity (1.09).

Sensitivity rankings for morphological and physiological traits: Table 2 indicates the relative sensitivity of 30 measured morphological and physiological traits in

Table 2: Relative sensitivity ranking for morphological and physiological traits of balansa clover in response to salinity

Morphological or physiological trait	Equation+	R^2	Significance level	Standard coefficient	Rank
Leaf Na^+/Ca^+ ratio	Y = 96.160+1.963×Salt	0.96	0.021	0.979	1
Fluorescence-old	Y = 99.82-0.030×Salt	0.95	0.026	-0.974	2
Leaf Na^+/K^+ ratio	Y = 97.62+0.579×Salt	0.93	0.033	0.967	3
Stem Na^+/Ca^+ ratio	Y = 92.66+0.924×Salt	0.93	0.034	0.966	4
Stem Na^+/K^+ ratio	Y = 89.66+1.901×Salt	0.89	0.056	0.944	5
Stem/Leaf Na	Y = 92.44-0.491×Salt	0.83	0.087	-0.913	6
Conductance-young leaves	Y = 101.94-0.868×Salt	0.82	0.092	-0.908	7
Transpiration rate-young leaves	Y = 108.16-0.734×Salt	0.70	0.162	-0.838	8
Leaf width	Y = 97.70-0.084×Salt	0.65	0.194	-0.806	9
Stem $\delta^{15}N$ ratio	Y = 65.620+1.440×Salt	0.53	0.274	0.726	10
Net photosynthesis-young leaves	Y = 112.24-0.495×Salt	0.503	0.291	-0.709	11
Xylem Na/Ca ratio	Y = 93.40+0.4471×Salt	0.44	0.339	0.661	12
Leaf total N	Y = 97.46-0.0667×Salt	0.42	0.350	-0.65	13
Leaf length	Y = 96.26-0.092×Salt	0.39	0.376	-0.624	14
Stem $\delta^{13}C$ ratio	Y = 100.240-0.006×Salt	0.38	0.383	-0.617	15
Plant height	Y = 90.58+0.205×Salt	0.37	0.389	0.611	16
Leaf $\delta^{15}N$ ratio	Y = 118.760-0.759×Salt	0.36	0.399	-0.601	17
Xylem Na^+/K^+ ratio	Y = 77.80+0.3964×Salt	0.30	0.450	0.55	18
Stem total N	Y = 95.70-0.0657×Salt	0.18	0.579	-0.421	19
Xylem Na	Y = 81.86+0.256×Salt	0.17	0.585	0.415	20
Transpiration rate-old leaves	Y = 131.70-0.446×Salt	0.13	0.633	-0.367	21
Net photosynthesis-old leaves	Y = 145.24-0.600×Salt	0.12	0.642	-0.358	22
Fluorescence-young	Y = 100.58-0.007×Salt	0.12	0.652	-0.348	23
Stomatal conductance-old leaves	Y = 142.20-0.491×Salt	0.10	0.681	-0.319	24
Stem total C	Y = 98.70+0.0057×Salt	0.03	0.840	0.16	25
Xylem K	Y= 103.16-0.047×Salt	0.02	0.871	-0.129	26
Shoot biomass (g pot^{-1})	Y = 80.240-0.063×Salt	0.01	0.887	-0.11	27
Leaf total C	Y = 98.90-0.0057×Salt	0.01	0.892	-0.1	28
Xylem Ca	Y = 103.52-0.092×Salt	0.01	0.903	-0.097	29
Leaf $\delta^{13}C$ ratio	Y = 99.92-0.0006×Salt	0.001	0.969	-0.031	30

+The Y-variables are expressed as (%) of the control treatment response to salinity. The rankings were developed using regression analysis and rankings were assigned based on the resulting absolute values of the standardized regression coefficients. The standardized coefficients allow variables to be compared irrespective of their scale of measurement. Based on this analysis, the top five most sensitive variables for balansa clover included leaf Na^+/Ca^{2+} ratio, chlorophyll fluorescence in older leaves, leaf Na^+/K^+ ratio, stem Na^+/Ca^{2+} ratio and stem Na^+/K^+ ratio in decreasing order of sensitivity. Furthermore, the top eight variables had R^2 values = 0.70 and indicate that the regression models were good enough for predictive purposes for these variables.

For burr medic the five most sensitive variables included leaf Na^+/Ca^{2+} ratio, stem Na^+/K^+ ratio, stem Na^+/Ca^{2+} ratio, xylem Na^+/Ca^{2+} ratio and leaf total N. Thus among these variables first three within the top five most sensitive variables for both burr medic and balansa clover. The R^2 values for all the five variables was 0.70 and therefore indicated that only these top five variables produced regression models that were good enough for predictive purposes. Therefore in screening for tolerance to saline conditions it is important to recognise the variation in the sensitivity of various morphological and physiological traits, because for the species in this study variables such as Na^+/K^+ ratios and Na^+/Ca^{2+} ratios in stems are quite sensitive to even low salt levels whereas growth and morphological traits as well as the leaf $\delta^{13}C$ ratios are among the least sensitive variables.

DISCUSSION

Salinity effects on growth and morphology: In general, when salinity stress occurs, plants become stunted as a result of reduced rate of leaf surface expansion and subsequent cessation of expansion (Parida and Das, 2005). However, the range over which such responses become evident varies from species to species and depends on whether a plant is a halophyte, salt-tolerant non-halophyte or salt-sensitive non-halophyte (Khan *et al*., 1999; Kurban *et al*., 1999; Aziz and Khan, 2001). For example, in a moderately salt tolerant non-halophyte such as *Alhalgi pseudoalhagi*, shoot biomass increased at 50 mM NaCl but decreased at 100 mM NaCl (Kurban *et al*., 1999). Present results indicate an unexpectedly higher plant height at 80 mM than at 20 and 40 mM. Furthermore, the lack of differences in the leaf widths, leaf lengths and shoot biomass yields among the control, 40 and 80 mM NaCl treatments indicate that these levels of salt imposed were generally not severe enough to cause substantial reductions in leaf surface expansion and thus lack of growth reduction for both balansa clover and burr medic.

Sodium, potassium and calcium accumulation in plant tissues: Theoretically, the Na^+/K^+ ratio in leaves has no lower limit under freshwater conditions, because Na^+ content can become infinitesimally small. However, plants under saline conditions take up Na^+ and thus the Na^+/K^+ ratio can be used as an indicator of either Na^+ or K^+ uptake. Further, the absolute Na and K levels can be used to distinguish plants that are Na^+ includers or Na^+ avoiders. In general, high levels of Na^+ in leaves indicate that plants are salt includes and if that is accompanied by low Na^+/K^+ ratio, indicate also high potassium uptake to balance the effects of excess Na^+. In contrast, Na^+ avoiders require less K^+ to achieve low Na^+/K^+ ratios and therefore would contain low Na^+ levels in the leaves (Asch *et al*., 2000). It is important to note that the metabolic activity of Na^+ is largely due to its ability to compete with K for binding sites essential for cellular function. More than 50 enzymes are activated by K^+ and Na^+ cannot be used as a substitute and thus high Na^+ or high Na^+/K^+ ratios can disrupt various enzymatic processes in the cytoplasm (Tester and Davenport, 2003).

The levels of Na^+ observed in this study were lower that those observed in tetraploid wheat selections (albeit under 150 mM NaCl treatment) and these ranged between 22-28 mg g^{-1} DW and the Na^+/K^+ ratio was between 1.37-1.67 (Munns and James, 2003). While the K^+ levels in both balansa clover and burr medic were comparable to those observed in wheat selections, the leaf Na^+ levels in the legumes were 25-42% of that reported for the wheat selections (Munns and James, 2003) implying that the relatively lower Na^+ accumulation in the former than the latter (*Triticum turgidum*).

High Na^+ concentrations also inhibit uptake and transport of Ca^{2+} such that plants that are able to take up more Ca^{2+} will have lower Na^+/Ca^{2+} ratios. Ca^{2+} plays a key role in protecting membrane integrity and in controlling the selectivity in ion uptake and transport. High Ca^{2+} levels has been reported to reduce membrane permeability to Na^+ and thus reduces accumulation of Na^+ via passive influx (Dasgan *et al*., 2002).

In a study conducted on *Atriplex griffithi* grown at 90 mM NaCl level, the Na^+ levels in leaves was 60 mg g^{-1} DW whereas that in stems was 28 mg g^{-1} DW and the Na^+/K^+ ratios were 29.2 and 18.1 for the leaves and stems, respectively (Khan *et al*., 1999). The Na^+ levels from this study, 6-9 times the levels observed from balansa clover and burr medic leaves and 3-4 times the levels observed in balansa clover and burr medic stems. This seems to indicate that the main strategy for salt tolerance in the legumes seem to be avoidance of high Na^+ accumulation, while at the same time accumulating larger quantities of K^+ and Ca^{2+} to negate the toxic effects of Na^+ and thus maintain a relatively low Na^+/K^+ ratio that is typical of glycophytes.

Table 3: Relative sensitivity ranking for morphological and physiological traits of burr medic in response to salinity

Morphological or physiological trait	Regression equation+	R^2	Significance level	Standard coefficient	Rank
Leaf Na^+/Ca^{2+} ratio	Y = 108.48+1.770×Salt	0.97	0.016	0.984	1
Stem Na^+/K^+ ratio	Y = 121.36+1.721×Salt	0.88	0.063	0.937	2
Stem Na^+/Ca^{2+} ratio	Y = 125.54+2.010×Salt	0.83	0.087	0.913	3
Xylem Na^+/Ca^{2+} ratio	Y = 107.44+0.511×Salt	0.79	0.113	0.886	4
Leaf total N	Y = 102.280- 0.216×Salt	0.72	0.149	-0.851	5
Leaf $\delta^{15}N$ ratio	Y = 127.680-1.203×Salt	0.61	0.221	-0.779	6
Stem/Leaf Na	Y = 106.66+0.236×Salt	0.59	0.235	0.765	7
Stem $\delta^{13}C$ ratio	Y = 99.60-0.016×Salt	0.52	0.281	-0.719	8
Stem $\delta^{15}N$ ratio	Y = 117.08-0.419×Salt	0.42	0.353	-0.647	9
Leaf total C	Y = 100.54-0.018×Salt	0.42	0.354	-0.646	10
Fluorescence-old leaves	Y = 99.64-0.057×Salt	0.41	0.361	-0.639	11
Fluorescence-young leaves	Y = 100.82+0.040×Salt	0.39	0.379	0.621	12
Net photosynthesis-old leaves	Y = 118.74-0.404×Salt	0.32	0.431	-0.569	13
Stem total N	Y = 101.18+0.050×Salt	0.28	0.476	0.525	14
Leaf Na^+/K^+ ratio	Y =108.38+0.209×Salt	0.19	0.559	0.441	15
Xylem K^+	Y= 99.64-0.053×Salt	0.16	0.606	-0.394	16
Plant height	Y = 103.18+0.040×Salt	0.13	0.639	0.361	17
Stomatal conductance-young leaves	Y = 65.86-0.336×Salt	0.11	0.662	-0.338	18
Xylem Ca^{2+}	Y = 206.70-1.279×Salt	0.11	0.669	-0.331	19
Stem total C	Y = 100.52+0.007×Salt	0.10	0.678	0.322	20
Transpiration rate-old leaves	Y = 141.06-0.399×Salt	0.08	0.719	-0.281	21
Stomatal conductance-old leaves	Y = 153.56-0.497×Salt	0.07	0.728	-0.272	22
Shoot biomass	Y = 108.56+0.052×Salt	0.05	0.779	0.221	23
Leaf $\delta^{13}C$ ratio	Y = 99.66+0.005×Salt	0.05	0.786	0.214	24
Transpiration rate-young leaves	Y = 68.72-0.164×Salt	0.03	0.816	-0.184	25
Net photosynthesis-young leaves	Y = 77.02-0.086×Salt	0.02	0.872	-0.138	26
Leaf length	Y = 98.54-0.010×Salt	0.01	0.882	-0.119	27
Xylem Na^+/K^+ ratio	Y = 125.00+0.089×Salt	0.01	0.882	0.118	28
Leaf width	Y = 93.30-0.008×Salt	0.01	0.922	-0.078	29
Xylem Na^+	Y = 125.36+0.045×Salt	0.003	0.942	0.058	30

+The Y-variables are expressed as (%) of the control treatment

It is clear from our study that Na^+ levels in the xylem are much lower than those in the leaves. The relatively similar Na^+/K^+ ratios in the xylem sap and the leaves indicate that the two legumes studied seem to be able to maintain a favourable Na^+/K^+ ratio throughout the plant. Other researchers have also reported relatively low Na^+ concentration in xylem sap of some non-halophytes that control Na^+ uptake in relatively moderate Na^+ levels similar to those imposed in this study (Chartzoulakis and Klapaki, 2000). In this study the sodium exclusion rates for balansa clover was 94% under 20 mM NaCl, 97% under 40 mM NaCl and 98% under 80 mM NaCl. For burr medic the exclusion rates were 91, 97 and 98%, under 20, 40 and 80 mM NaCl treatments, respectively. These results indicate that for the salt levels up to 80 mM the salt exclusion mechanism is dominant in controlling Na^+ levels in the xylem.

Relative sensitivity and conclusions: The relative order of sensitivity of morphological and physiological traits indicate that at low salt levels plants take up Na^+ but do not suffer from osmotic stress and therefore no substantial yield reduction occurs. This is indicated by the results in Table 2 and 3 which indicate that Na^+ accumulation in both balansa clover and burr medic leaves and stems occurs even at low salt levels with corresponding increase in the Na^+/K^+ ratio. Despite these changes the two legumes seem to have adapted well and managed to assimilate CO_2 and maintain their transpiration (Table 3).

These studies indicate that despite the difficulties associated with determination of leaf and stem Na^+ contents and K^+ contents, these parameters are the most important indicators of salinity tolerance and would be affected before salt stress effects on yield, chlorophyll fluorescence, net photosynthesis, stomatal conductance, transpiration rates, 13C discrimination and 15N discrimination become evident.

REFERENCES

ANZECC, 2001. Implications of salinity for biodiversity conservation and management. Report prepared for the Australian and New Zealand environment and conservation council by a task force established by the standing committee on conservation. Environment Australia, Canberra. http://www.environment.sa.gov.au/biodiversity/pdfs/salinity_bio.

Asch, F., M. Dingkuhn, K. Dörfflin and K. Miezan, 2000. Leaf K/Na ratio predicts salinity-induced yield loss in irrigated rice. Euphytica, 113: 109-118.

Aziz, I. and M.A. Khan, 2001. Experimental assessment of salinity tolerance of Ceriops tagal seedlings and saplings from the Indus delta, Pakistan. Aqua. Bot., 70: 259-268.

Bell, D.T., 1999. Australia trees for the rehabilitation of waterlogged and salinity-damaged landscapes. Aust. J. Bot., 47: 697-716.

Chartzoulakis, K. and G. Klapaki, 2000. Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages. Sci. Hortic., 86: 247-260.

Dasgan, H.Y., H. Aktas, K. Abak and I. Cakmak, 2002. Determination of screening techniques to salinity tolerance in tomatoes and investigation of genotype responses. Plant. Sci., 163: 695-703.

Dear, B.S., P.S. Cocks, E.C. Wolfe and D.P. Collins, 1998. Established perennial grasses reduce the growth of emerging subterranean clover seedlings through competition for water, light and nutrients. Aust. J. Agric. Res., 49: 41-51.

Gorham, J., 1996. Mechanisms of salt tolerance of halophytes. In: Halophytes and Biosaline Agriculture. Redouane, C.A., C.V. Malcolm and A. Hamdy (Eds.). Marcel Dekker Inc., New York, pp: 31-53.

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

Jacoby, B., 1994. Mechanisms Involved in Salt Tolerance by Plants. In: Handbook of Plant and Crop Stress. In: Pessakakli, M. (Ed.). Marcel Dekker, New York, pp: 97-125.

Khan, M.A., I.A. Ungar and A.M. Showalter, 1999. Effects of salinity on growth, ion content and osmotic relations in *Halopyrum mocoronatum* (L.) Stapf. J. Plant Nutr., 22: 191-204.

Kozlowski, T.T., 1997. Responses of woody plants to flooding and salinity. Tree Physiol. Monograph, 1: 1-29.

Kurban, H., H. Saneoka, K. Nehira, R. Adilla, G.S. Premachandra and K. Fujita, 1999. Effect of salinity on growth, photosynthesis and mineral composition in a leguminous plant *Alhagi pseudoalhagi* (Bieb.). Soil Sci. Plant Nutr., 45: 851-862.

Munns, R. and R.A. James, 2003. Screening methods for salinity tolerance: A case study with tetraploid wheat. Plant Soil, 253: 201-218.

Parida, A.K. and A.B. Das, 2005. Salt tolerance and salinity effects on plants: A review. Ecotoxic. Environ. Safety, 60: 324-349.

Rogers, M.E., A.D. Craig, R.E. Munns, T.D. Colmer, P.G.H. Nichols, C.V. Malcolm, E.G. Barrett-Lennard, A.J. Brown ***et al.***, 2005. The potential for developing fodder plants for salt-affected areas of southern and eastern Australia: An overview. Aust. J. Exp. Agric., 45: 301-329.

SAS Institute Inc., 2000. Statistical Analysis System. 1st Edn., SAS Institute Inc., Jackknife and Bootstrap, New York: Springer-Verlag .

Tester, M. and R. Davenport, 2003. Na^+ tolerance and Na^+ transport in higher plants: A review. Ann. Bot., 91: 503-527.

Ulrich, S., 1998. Xylem sap sampling-new approaches to an old topic. Trends Plant Sci., 3: 293-298.