

INFLUENCE OF SALINITY ON GROWTH AND OSMOTIC RELATIONS OF *SPOROBOLUS IOCLADOS*

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Abstract

Sporobolus ioclados (Nees ex Trin.) Nees (Poaceae) is a perennial salt secreting grass distributed from coastal sand dunes and marshes of the Arabian Sea to saline flats throughout the Indus basin in Pakistan. Effects of NaCl on growth, water relations and ion accumulation were studied. Plants were grown in 0, 100, 200, 300, 400 and 500 mM NaCl in sand culture using sub-irrigation method. Fresh and dry weight of roots and shoots were highest in non-saline control. Increase in salinity inhibited growth and plants had high mortality at 500 mM NaCl. Tissue water decreased with an increase in salinity. Water and osmotic potential decreased with increase in salinity and plants lost turgor. Stomatal conductance progressively decreased with the increase in salinity. Shoot ion content was low and showed little variation with increase in salinity.

Introduction

Salt tolerance of halophytic grasses varies with the ecotype, species, habitat and other environmental factors (Gulzar *et al.*, 2003ab). Grasses like *Aeluropus lagopoides* and *Urochondra setulosa* could survive in up to 1000 mM NaCl (Bodla *et al.*, 1995; Gulzar *et al.*, 2003ab) while a number of them could survive in salinity (550 to 600 mM NaCl) approaching seawater (Glenn, 1987; Hester *et al.*, 1996, 2001). Some grasses could grow in the soil salinity ranges between 300 to 500 mM NaCl (Mahmood *et al.*, 1996; Bell & O'Leary, 2003; Peng *et al.*, 2004) while others could not survive in the salt concentration above 300 mM NaCl (La Peyre & Row, 2003; Khan *et al.*, 1999).

Mechanisms of salt tolerance are of two main types: those minimizing the entry of salts into the plant and those minimizing the concentration of salt in the cytoplasm (Munns, 2002). Halophytic grasses have both types of mechanisms; they exclude salt well and use water loss to concentrate solutes for osmotic adjustment (Glenn, 1987). Monocotyledonous halophytes generally have much lower water content, Na⁺: K⁺ ratios and mineral content than dicotyledonous halophytes growing at the same location (Gorham *et al.*, 1980; Glenn, 1987). Sodium exclusion method of salt tolerance appears less efficient than sodium accumulation particularly in the succulent xerophytes (Wang *et al.*, 2004). At higher salinity, *Sporobolus arabicus* accumulated more Na⁺ in comparison to other species studied (Mahmood *et al.*, 1996). In *Halopyrum mucronatum*, accumulation of Na⁺ and Cl⁻ increased with increasing salinity, while K⁺, Ca²⁺ and Mg²⁺ decreased (Khan *et al.*, 1999).

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Ion ratios could be helpful in categorizing the physiological response of a plant (salt-excluding, salt-secreting or salt-diluting) in relation to ion selectivity under increasing substrate salt concentrations (Wang *et al.*, 2002). Substantial differences in Na^+ and K^+ accumulation between salt-resistant species may be due to differences in the selective ion transport capacities at root level (Wang *et al.*, 2002). Salt secreting species would be expected to have the weakest selective transport capacity for K^+ over Na^+ as most of the salt would have to be transported up to the stem and excluded from the leaf via salt glands. *Aeluropus lagopoides* did show high selectivity for K by retaining greater amounts of Cl^- and Mg^{2+} in root than in shoots (Gulzar *et al.*, 2003a), while *Urochondra setulosa* shoots did not show high K selectivity (Gulzar *et al.*, 2003b). Salinity induced inhibition of plant growth may occur due to the effects of high Na^+ , Cl^- or SO_4^{2-} by decreasing the uptake of essential elements such as P, K, NO_3 and Ca, ion toxicity or osmotic stress (Zhu, 2001, 2002).

Sporobolus ioclados (Nees ex Trin.) Nees (Poaceae) (syn: *Sporobolus arabicus*) is a stoloniferous perennial grass with a wide range of distribution extending from the sand dunes and marshes of the Arabian sea coast to the salt flats and saline deserts in northern Pakistan (Cope, 1982). Aerial shoots sprout from the stoloniferous base after considerable monsoon rains and set seeds late in the fall. However, new individuals are recruited from seeds in less saline and dry dune habitats, whereas, recruitment through stolons is common in salt marshes. *Sporobolus ioclados* populations are distributed in saline wet patches and dry sandy areas in Karachi University campus where salinity varies from 200 to 400 mM NaCl (Khan, 1993). *Sporobolus ioclados* seeds could germinate in up to 500 mM NaCl, however, exposure to high salinity and temperature caused loss of seed viability (Gulzar & Khan, 2003). *Sporobolus ioclados* is a good source of fodder for cattle and could be cultivated with saline water sources for increasing productivity of salinized soils. This study was conducted to determine the effects of salinity on growth, water relations and ion accumulation of *S. ioclados* at the mature vegetative phase of life cycle.

Materials and Methods

Seeds of *S. ioclados* were collected during the winter of 2000 from inland salt marshes located at University of Karachi campus, Pakistan. Seeds were separated from the inflorescence and stored at 4°C and growth studies were started immediately in an open-air green house located at University of Karachi. Seeds were surface sterilized using sodium hypochlorite. Seeds were germinated in 10 cm x 8 cm plastic pots filled three fourths with sandy soil. Plants were raised on half strength Hoagland and Arnon solution No. 2 for two weeks until they were 1 cm in height. Plants were thinned to five similar sized plants in each pot. A half-strength Hoagland and Arnon solution no. 2 nutrient solution was used to supply the macronutrients and micronutrients. Pots were sub-irrigated, and the water level was adjusted daily to correct for evaporation. Salt solutions were completely replaced once a week to avoid build-up of salinity in pots. Six salinity treatments viz., 0, 100, 200, 300, 400, and 500 mM NaCl were employed after a preliminary test of salinity tolerance. Salinity levels were raised gradually at daily intervals. Plants were grown under saline conditions for 6 weeks after maximum salinity was achieved. At the end of the experiment, plants were harvested and fresh and dry weight of stem and root, shoot and root length, number of leaves and tillers were recorded. Plants were oven-dried at 80°C for 48 h before dry weight was determined.

Water potential was measured on punched disks from randomly chosen leaves in a C-52 chamber with the help of a HR-33 dew point micro-voltmeter (Wagtech). Press sap technique was used for measuring leaf osmotic potential. Leaf turgor pressure was estimated from the difference between leaf osmotic and water potentials. Leaf stomatal conductance was measured with the help of AP-4 Porometer (Delta-T Devices). Chloride ion was measured with a Beckman specific ion electrode. Cation content of plant root and shoot parts was analysed using a Perkin Elmer model 360 atomic absorption spectrophotometer. The Na^+ and K^+ levels of plant were examined by flame emission spectrometry and Ca^{2+} and Mg^{2+} levels by atomic absorption spectrometry.

A completely randomized ANOVA analysis was used to test for significant differences within mean values for growth, water relations and ion relations. A Bonferroni test was carried out to check for differences within individual treatment means (Anonymous, 2002).

Results

A one-way ANOVA on the growth of *S. ioclados* indicated that salinity inhibited shoot dry weight ($F = 10.17$, $P < 0.001$), shoot fresh weight ($F = 13.43$, $P < 0.001$), shoot length ($F = 96.5$, $P < 0.001$), root length ($F = 39.14$, $P < 0.001$) and number of leaves ($F = 8.55$, $P < 0.001$). Plant biomass and all other growth parameters mentioned above decreased with the increase in salinity (Table 1).

A one-way ANOVA indicated that salinity significantly affected tissue water content of *S. ioclados* on a unit dry weight basis ($F = 10.16$, $p < 0.001$) and water per plant basis ($F = 4.66$, $p < 0.001$). Succulence, when expressed as g tissue water g^{-1} dry weight basis or as tissue water per plant decreased at higher salinities (Table 2). Water potential ($R^2 = 0.91$, $p < 0.0001$) and osmotic potential ($R^2 = 0.91$, $p < 0.0001$) increased with increase in salinity (Fig. 1). Turgor ($R^2 = 0.45$, $p < 0.01$) decreased with the increase in salinity (Fig. 1). Stomatal conductance ($R^2 = 0.92$, $p < 0.0001$) substantially decreased with the increase in salinity (Fig. 1).

A two-way ANOVA indicated that salinity and plant parts and their interactions had a significant effect on the ion content of both roots and shoots (Table 3). Sodium, chloride and calcium remained unchanged in the shoot while potassium and magnesium decreased with the increase in salinity (Table 4). Sodium content of roots increased substantially with the salinity while there was little increase in chloride. Potassium, calcium and magnesium decreased in root tissues with the increase in salinity (Table 4). Ash content did not change substantially with the change in salinity in both root and shoot. Ash content of shoot was lower (12 %) in comparison to (35%) root (Table 4). Na:K ratio changed little in shoot but increased in the root (Fig. 2). Na:Ca ratio increased at low salinity in shoot and at high salinity in root (Fig. 2). K:Ca ratio decreased in shoot and there was no effect in root. Na:Cl ratio did not change in shoot but substantially increased in low salinity and declined with a further increase in salinity (Fig. 2).

Shoot to root ratio for K^+ , Cl^- and Mg^{++} increased at low salinity and decreased with a further increase in salinity (Fig. 3). Shoot to root ratio for Na^+ progressively decreased and for Ca^{++} and Ash it progressively increased with the increase in salinity.

Table 1. Effect of salinity on the growth parameters of *Sporobolus ioclados*.

| NaCl (mM) | Growth parameters of <i>Sporobolus ioclados</i> | | | | | | | | | |
|-----------|---|----------------------------|----------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|----------------------------|--|--|
| | Shoot fresh weight (g) | Shoot dry weight (g) | Root fresh weight (g) | Root dry weight (g) | Shoot length (cm) | Root length (cm) | No. of leaves | No. of tillers | | |
| 0 | 1.71 ^a ±0.33 | 1.09 ^a ±0.18 | 1.13 ^a ±0.12 | 0.77 ^a ±0.14 | 61.83 ^a ±3.17 | 26.0 ^a ±2.65 | 15.67 ^a ±4.26 | 7.33 ^a ±1.86 | | |
| 100 | 1.27 ^a ±0.18 | 0.71 ^b ±0.14 | 0.73 ^b ±0.18 | 0.33 ^b ±0.03 | 42.83 ^b ±3.35 | 11.83 ^b ±0.93 | 5.00 ^b ±0.58 | 2.0 ^b ±0 | | |
| 200 | 1.00 ^a ±0.19 | 0.66 ^b ±0.17 | 0.34 ^c ±0.03 | 0.24 ^b ±0.03 | 14.00 ^c ±3.51 | 6.67 ^c ±0.17 | 4.33 ^b ±0.88 | 2.0 ^b ±0.58 | | |
| 300 | 0.67 ^b ±0.12 | 0.51 ^b ±0.1 | 0.18 ^d ±0.02 | 0.11 ^c ±0.0 | 6.67 ^d ±2.33 | 4.67 ^c ±1.67 | 2.33 ^c ±0.33 | 0 ^c ±0 | | |
| 400 | 0.32 ^c ±0.05 | 0.24 ^c ±0.03 | 0.1 ^e ±0.01 | 0.08 ^c ±0.01 | 2.17 ^d ±0.44 | 4.67 ^c ±0.93 | 2.33 ^c ±0.33 | 0 ^c ±0 | | |
| 500 | 0.08 ^d ±0.02 | 0.07 ^d ±0.02 | 0.03 ^e ±0 | 0.02 ^c ±0 | 1.00 ^d ±0 | 2.83 ^d ±0.17 | 1.67 ^c ±0.33 | 0 ^c ±0 | | |

Values are means (±S.E.). Different letters in superscript represent significant ($p < 0.05$) differences between salinity treatments. ANOVA, Bonferroni test.

Table 2. Effect of salinity on tissue water content of *Sporobolus ioclados*.

| NaCl (mM) | Tissue water (g plant ⁻¹) | Tissue water (g g ⁻¹ dry wt.) |
|-----------|---------------------------------------|--|
| 0 | 0.61 ^a ± 0.29 | 0.57 ^a ± 0.23 |
| 100 | 0.56 ^a ± 0.08 | 0.78 ^a ± 0.18 |
| 200 | 0.34 ^b ± 0.03 | 0.52 ^a ± 0.11 |
| 300 | 0.16 ^c ± 0.03 | 0.31 ^b ± 0.06 |
| 400 | 0.08 ^d ± 0.02 | 0.33 ^b ± 0.12 |
| 500 | 0.01 ^d ± 0.01 | 0.14 ^c ± 0.05 |

Different letters in superscript represent significant ($p < 0.05$) differences between salinity treatments (Bonferroni).

Table 3. Results of two-way analysis of variance of characteristics by salinity (S) and plant part (P).

| Dependent variable | Salinity (S) | Plant part (P) | P x S |
|--------------------|--------------|----------------|---------|
| Sodium | 8.3*** | 60.2*** | 10.0*** |
| Potassium | 74.7*** | 300.7*** | 44.3*** |
| Calcium | 37.7*** | 186.7*** | 40.1*** |
| Magnesium | 21.0*** | 6.1* | 13.5*** |
| Chloride | 15.8*** | 20.3*** | 8.3*** |

Numbers are F-values significant at * $p < 0.01$, *** $p < 0.0001$.

Discussion

Sporobolus ioclados is one of the most common halophytic grasses found both in the coastal salt marshes and deserts as well as inland saline deserts around Karachi, and other regions of Pakistan. The inland salt flats with a high water table and high salinity are often dominated by *Sporobolus ioclados* in association with *Suaeda fruticosa* and *Haloxylon stocksii*. *Sporobolus ioclados* showed rapid growth in non-saline medium and plants attained maximum height and weight in only 20 d as compared to 45 d for *Aeluropus lagopoides* and 60 d for *Urochondra setulosa* (Gulzar *et al.*, 2003 ab).

Sporobolus ioclados barely survived 500 mM NaCl and therefore, appears to be moderately salt tolerant in comparison to other salt secreting grass species growing in the same habitat. *Halopyrum mucronatum*, *Aeluropus lagopoides* and *Urochondra setulosa*, the other dominant grasses showed a variable response to salinity (Khan *et al.*, 1999; Gulzar *et al.*, 2003ab). *Aeluropus lagopoides* and *Urochondra setulosa* could survive 1000 mM NaCl but showed 50% mortality while other grasses like *H. mucronatum* suffered high mortality at 300 mM NaCl (Khan *et al.*, 1999). Growth of *S. ioclados* is inhibited with an increase in salinity. Most halophytic grasses do not survive in more than 300 mM NaCl (Glenn, 1987). Optimal growth of some monocotyledonous halophytes was observed in 300 mM NaCl (Breen *et al.*, 1977; Naidoo & Mundree, 1992; Marcum & Murdoch, 1994; Marcum, 1995; Lissner & Schierup, 1997). Glenn (1987) reported that grasses *viz.*, *Aeluropus*, *Paspalum*, *Puccinellia*, *Spartina* and *Sporobolus* from intertidal zones survived 540 mM NaCl while those from brackish habitat were not as tolerant. Greipsson & Davy (1996) also reported differences in salt tolerance of *Leymus arenarius* seedlings grown with seeds from inland and coastal populations. Seedlings of coastal origin had higher number of tillers at 200-400mM NaCl, while dry matter production was less adversely affected at higher salinities. Optimal growth of *Sporobolus virginicus* was observed at salinity levels between 100-150 mM NaCl (Bell & O'Leary, 2003).

Table 4. Ionic content (mmol g⁻¹ dry wt.) of *Sporobolus ioclados* grown in different salinity concentrations.

| NaCl (mM) | Sodium | | Potassium | | Calcium | | Magnesium | | Chloride | | % Ash | |
|--------------|--------------------|--------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------------------|--------------------|
| | Shoot | Root | Shoot | Root | Shoot | Root | Shoot | Root | Shoot | Root | Shoot | Root |
| 0 | 7.96 ^a | 5.50 ^a | 3.32 ^a | 1.34 ^a | 0.34 ^a | 9.56 ^a | 1.14 ^a | 0.94 ^a | 0.94 ^a | 0.32 ^a | 11.20 ^a | 43.90 ^a |
| | ±0.50 | ±1.27 | ±0.28 | ±0.01 | ±0.05 | ±0.48 | ±0.03 | ±0.07 | ±0.19 | ±0.05 | ±0.38 | ±6.65 |
| 100 | 10.54 ^a | 18.62 ^b | 2.09 ^b | 0.49 ^b | 0.35 ^a | 2.60 ^b | 1.32 ^a | 0.38 ^b | 1.31 ^a | 0.55 ^b | 12.63 ^a | 35.97 ^a |
| | ±1.42 | ±4.89 | ±0.01 | ±0.08 | ±0.03 | ±0.72 | ±0.26 | ±0.02 | ±0.12 | ±0.15 | ±0.53 | ±8.23 |
| 200 | 18.28 ^a | 28.70 ^b | 2.38 ^b | 0.34 ^b | 0.30 ^a | 3.41 ^b | 0.32 ^b | 0.45 ^b | 1.56 ^a | 0.17 ^a | 11.70 ^a | 36.60 ^a |
| | ±6.36 | ±7.24 | ±0.04 | ±0.03 | ±0.01 | ±0.64 | ±0.01 | ±0.04 | ±0.24 | ±0.04 | ±0.31 | ±5.11 |
| 300 | 4.36 ^b | 19.05 ^b | 0.85 ^c | 0.74 ^c | 0.58 ^b | 1.64 ^c | 0.48 ^b | 0.76 ^a | 2.13 ^a | 3.17 ^c | 18.50 ^b | 37.80 ^a |
| | ±0.36 | ±3.85 | ±0.07 | ±0.01 | ±0.01 | ±0.32 | ±0.04 | ±0.07 | ±0.65 | ±0.24 | ±0.35 | ±3.08 |
| 400 | 3.05 ^b | 52.09 ^c | 0.83 ^c | 0.72 ^c | 0.51 ^b | 1.10 ^c | 0.43 ^b | 0.46 ^b | 2.01 ^a | 1.76 ^a | 12.97 ^a | 20.53 ^b |
| | ±0.30 | ±8.32 | ±0.03 | ±0.03 | ±0.05 | ±0.26 | ±0.03 | ±0.03 | ±0.31 | ±0.21 | ±0.95 | ±3.04 |
| 500 | 9.24 ^a | 44.95 ^c | 0.81 ^c | 0.57 ^b | 0.35 ^a | 1.30 ^c | 0.41 ^b | 0.39 ^b | 3.15 ^b | 0.74 ^b | 17.10 ^b | 20.83 ^b |
| | ±0.84 | ±3.97 | ±0.05 | ±0.05 | ±0.03 | ±0.49 | ±0.03 | ±0.03 | ±0.41 | ±0.17 | ±0.93 | ±3.00 |

Different letters in superscript over means (±S.E.) represent significant differences between salinity treatments, Bonferroni test.

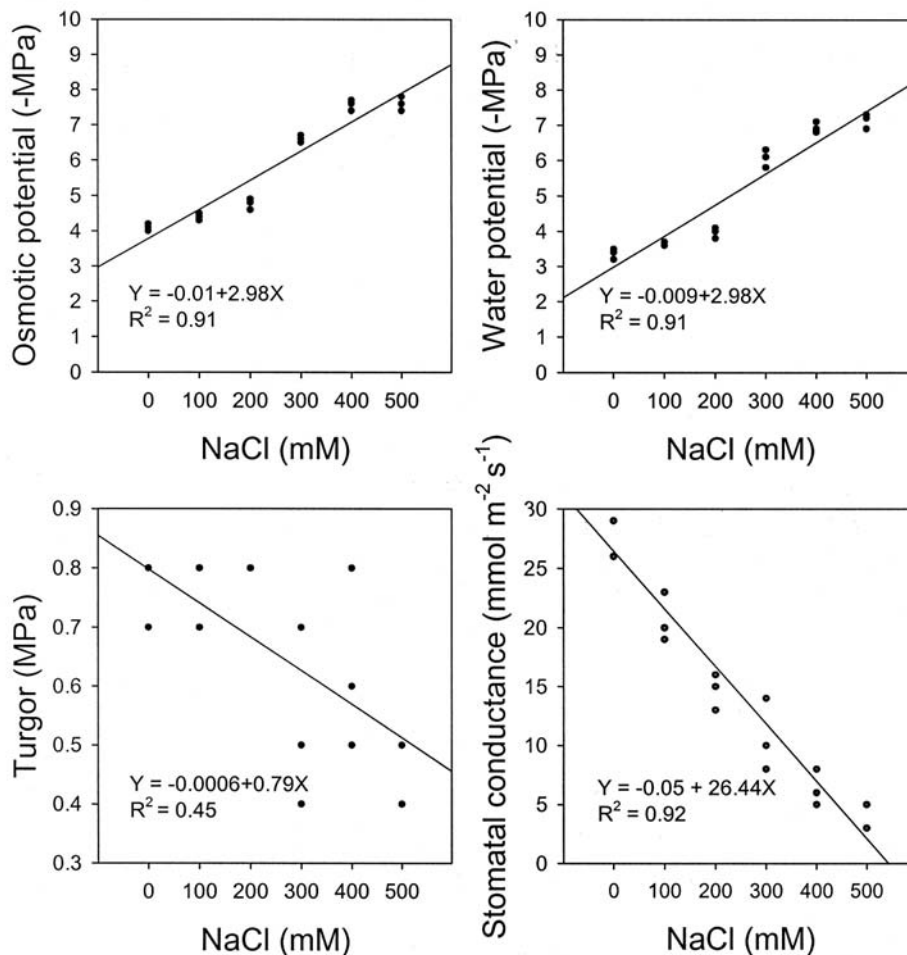


Fig. 1. Effect of NaCl salinity (0, 100, 200, 300, 400 and 500 mM) on the water potential, osmotic potential, pressure potential and stomatal conductance of *Sporobolus ioclados*. A linear regression is represented.

Succulence, when expressed as $\text{g tissue water g}^{-1}$ dry weight decreased at higher salinities. Similarly, tissue water per plant basis decreased with the increase in salinity. This indicates that high salinity caused a reduction in the total water content and eventually the growth of plants. Glenn (1987) reported that water content of 19 grasses declined with the increase in salinity. Similar results were shown by Marcum & Murdoch (1990) in 11 grasses. Succulence in perennial grasses from Karachi, Pakistan showed a variable pattern. Succulence increased in *H. mucronatum* (Khan *et al.*, 1999), while it decreased with increase in salinity in *U. setulosa* and *A. lagopoides* (Gulzar *et al.*, 2003ab). This decrease in succulence with salinity could be attributed to low ion accumulation in the shoot tissue, as there was little variation in ash content with the increase in salinity. Plants maintain their osmotic balance by reducing the tissue water

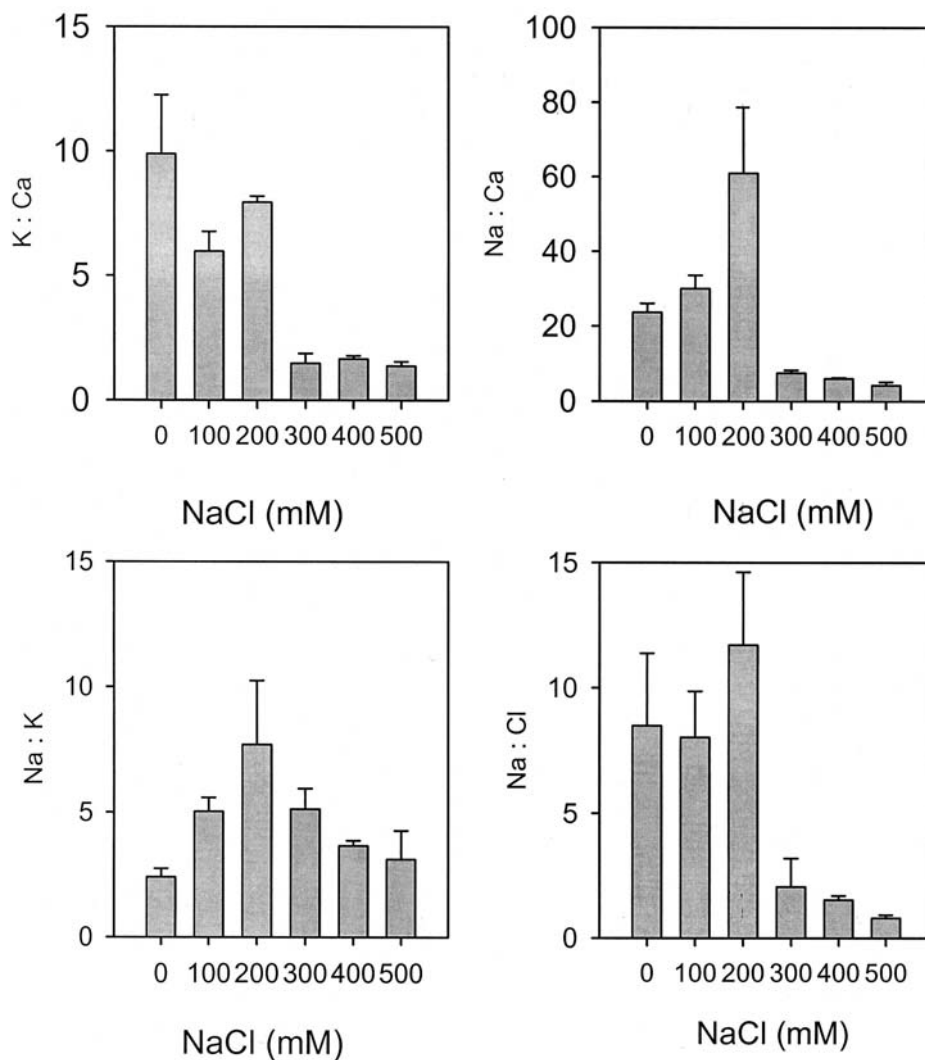


Fig. 2. Effect of NaCl salinity (0, 100, 200, 300, 400 and 500 mM) on the Na: K, Na: Ca, K: Ca and Na: Cl ratios of *Sporobolus ioclados*.

rather than by sodium uptake (Glenn, 1987). *Sporobolus ioclados* showed a sharp drop in the water and osmotic potentials and could not maintain turgor with increasing salinity. Stomatal conductance was reduced and to avoid water loss resulting in lower biomass production. Reduced growth in perennial plants would enhance survival under saline conditions due to lack of interspecific competition with the less tolerant glycophytic species.

Sporobolus ioclados plants seem to exercise a greater control on the ion movement by preventing major ions like Na^+ and Cl^- from entering the shoot with the increase in substrate salinity. Ions like Na^+ , Ca^{2+} and Cl^- remained unchanged in shoot with the

increase in salinity while K^+ and Mg^{2+} decreased. However, in roots the amount Na^+ and Cl^- increased with the increase in salinity. Shoot to root ratio of Na^+ decreased with the increase in salinity indicating that the absorbed sodium is predominantly stored in roots. Whereas, shoot to root ratio of Cl^- , Ca^{2+} and Mg^{2+} remained unchanged with the increase in salinity. However, shoot to root ratio of K^+ increased in lower salinity and substantially decreased in higher salinities indicating active transfer of K^+ to the shoot. Plant species vary widely in their ability to translocate sodium to the shoot (White & Broadley, 2003; Subbarao *et al.*, 2003; Tester & Devenport, 2003). Plants that take up considerable amounts of Na^+ , mostly retaining it in root with relatively little translocation to shoot are termed as nitrophobes. Bell & O'Leary (2003) did not find accumulation of sodium in leaves with increasing nutrient medium salinity from 125-450 mM NaCl, probably due to the increased secretion from leaves. *Spartina alterniflora* did not display a significant difference in leaf xylem pressure and total cation concentrations between populations of different salinity tolerance (Hester *et al.*, 2001). It is well documented that *S. alterniflora* can exert a considerable control over ion accumulation via selective processes of ion exclusion and secretion of Na^+ over K^+ (Bradley & Morris, 1991). Similar control was also reported for *Aeluropus lagopoides* (Gulzar *et al.*, 2003a). However, Na^+ and Cl^- accumulated in shoots of other grasses such as *Halopyrum mucronatum*, *Sporobolus spicatus* and *Urochondra setulosa* with increasing soil salinity (Khan *et al.*, 1999; Ramdan, 2001; Gulzar *et al.*, 2003b).

Ion ratios could be helpful in categorizing the physiological response of a plant (salt-excluding, salt-secreting or salt-diluting) in relation to ion selectivity under increasing substrate salt concentrations (Wang *et al.*, 2002). Substantial differences in Na^+ and K^+ accumulation between salt-resistant species may be due to differences in the selective ion transport capacities at root level (Wang *et al.*, 2002). Na^+ : K^+ ratios in leaves were lower than in roots indicating discrimination in Na^+ uptake in roots. Glenn (1987) studied the effect of salinity on the growth of 14 grasses and measured ash and cations. He reported that in response to salt stress, Na^+ in shoots increased, K^+ decreased, Mg^{2+} and Ca^{2+} remained about the same and water content decreased. Grasses differed in numerous ways from dicotyledonous halophytes (Glenn & O'Leary, 1984). They had much lower Na : K ratios (1.99 : 1 versus 11.8 : 1) lower mineral content (16 g dry wt⁻¹) and lower water contents (3 versus 6 g dry wt⁻¹) when grown on 180 mM NaCl. This supports the concepts of a monocot 'physiotype' for salt tolerance (Albert & Popp, 1977; Gorham *et al.*, 1980). The molalities of the shoot tissue at 180 mM were similar for monocots and dicotyledonous euhalophytes (943 versus 874 mmol kg⁻¹) (Glenn & O'Leary, 1984; Glenn, 1987). Glenn (1987) suggested that grasses maintain osmotic balance by water loss rather sodium uptake. Gorham *et al.*, (1980) reported high K^+ and low Na^+ content in 20 monocotyledonous species and the results were in agreement with previous reports (Albert & Popp, 1977). However, species like *Spartina anglica*, *Triglochin maritima* and *Zostera maritima* accumulated high amounts of sodium (Albert & Popp, 1977; Storey *et al.*, 1977).

Sporobolus ioclados is a fast growing, moderately salt tolerant grass, which could survive salinity stress by keeping low ash and water content in leaves to maintain a favorable osmotic balance. This grass is used locally as a fodder for livestock and could be useful in coastal sand dune stabilization. It is currently being grown experimentally in the field as a fodder using brackish water irrigation.

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