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Physiological and biochemical aspects of tolerance of three grass species to varying Na^+/Ca^{2+} ratios

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Abstract

The effects of decreasing Ca^{2+} concentrations (Na/Ca ratios were 24, 49, 99 and 199) of the saline growth medium (NaCl concentration 200 mM) on three grass species *Cenchrus pennisetiformis* Hochst. & Steud., *Leptochloa fusca* L. Kunth. and *Panicum turgidum* Forssk. were assessed after 7 weeks growth in sand culture.

L. fusca produced the highest dry biomass of all the species at varying Na/Ca ratios. Number of tillers per plant and number of green leaves per tiller were reduced significantly only in *C. pennisetiformis*.

Leaf water potential of *C. pennisetiformis* decreased at all external Na/Ca ratios, whereas in *L. fusca* it decreased only at an Na/Ca ratio of 99. Leaf osmotic potential of *L. fusca* consistently decreased at all Na/Ca ratios, whereas that of the other two species remained unchanged. The shoot and root total sugars of all species remained unaffected at all decreasing Ca^{2+} concentrations. In *P. turgidum* chlorophyll *b* and total chlorophyll decreased consistently at all Na/Ca ratios, but in *L. fusca* they decreased only at the highest Na/Ca ratios. The leaf soluble proteins of all the species remained unaffected at all Na/Ca ratios. The leaf free amino acids decreased significantly in *L. fusca* with the increase in Na/Ca ratios. The leaf proline content was only decreased in *L. fusca* at the highest Na/Ca ratio. The significant correlations between the growth of the three grass species and other variables determined in this study were not found.

Introduction

Cenchrus pennisetiformis Hochst. & Steud., and *Panicum turgidum* Forssk. are among the valuable forage grasses in arid regions (Ashraf and Bokhari 1987). *Leptochloa fusca* L. Kunth. is a perennial grass which is usually grown under high salinity.

It has been recently reported that adequate levels of calcium can alleviate the adverse effects of NaCl salinity on plant growth. The role of calcium in the proper functioning of biological membranes has also been reported (*e.g.* Leopold and

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Willing 1984). Kawasaki and Moritsugu (1978) and Maas and Grieve (1987) found that the decrease in Na/Ca ratio in the medium slightly increased the growth of rice and sorghum, respectively. Yeo and Flowers (1985) did not find any effect of varying Na/Ca ratios in culture solution on the growth of rice. Similarly Kent and Läuchli (1985) found that addition of supplemental Ca^{2+} to the medium did not improve germination of cotton.

The present study was undertaken to investigate the effect of varying Na/Ca ratios on the growth of three grass species particularly in relation to different physiological/biochemical parameters such as water relations, or soluble proteins, total sugars, proline, and total free amino acids. The organic osmotics have long been known to play crucial role in osmotic adjustment and hence maintenance of plant growth under salinity stress (Flowers *et al.* 1977, Greenway and Munns 1980, Wyn Jones 1980). Therefore, one of the major objectives of the present study was to draw parallels between growth and accumulation of different organic solutes in response to varying Na/Ca ratios in the growth medium.

Materials and methods

Seeds of two grass species, *Cenchrus pennisetiformis* Hochst. and Steud. and *Panicum turgidum* Forssk. were collected from the Cholistan desert of Pakistan, whereas those of *Leptochloa fusca* L. Kunth. from a field near the Botanic Garden of the Bahauddinn Zakariya University, Multan. Plants were grown in a greenhouse at 30 ± 3 °C day temperature and 16 ± 2 °C night temperature in pots with sand and nutrient solution. The varying Na/Ca ratios prepared are given below:

Treatment*	Na/Ca ratio [mM]	NaCl [mM]	$Ca(NO_3)_2.4H_2O$ [mM]	$CaCl_2.2H_2O$ [mM]
T_1	24	192	4	4
T_2	49	196	4	-
T_3	99	198	2	-
T_4	199	199	1	-

Full strength Hoagland nutrient solution (T_0) served as control.

Salt treatments in full strength Hoagland nutrient solution were begun 50 d after the start of the experiment and salt treatment was increased stepwise, in aliquots of 50 mM on alternate days. Seven weeks after the salt treatment various physiological and biochemical parameters were measured. Leaf diffusive resistance was measured with an automatic porometer (*Mk3 Delta-T*) at 09.00, 12.00, and 16.00 (local time). For the determination of leaf osmotic potential, a fully expanded youngest leaf was excised and frozen in 2.0 cm^3 polypropylene tubes for two weeks after which time it was thawed, and the sap was extracted by crushing the material with a metal rod. After centrifugation (8000 *g*) for 4 min, the sap was used directly for osmotic potential determination in an osmometer *TP 10B* (*Camlab Limited*). For the measurement of leaf water potential a fully expanded leaf was detached from each plant at 09.00. The leaf water potential measurements were made with a pressure bomb (*Chas. W. Cook & Sons,* Birmingham, U.K.) Chlorophyll content was estimated spectrophotometrically by the method described by Witham *et al.* (1971). Proline was estimated spectrophotometrically following the ninhydrin method described by Bates *et al.* (1973), using pure proline (*Merck*) as a standard. Soluble proteins were estimated as described by Lowry *et al.* (1951). Free amino acids were determined following the method described by Hamilton and Van Slyke (1943). Total sugars were estimated as described by Ashwell (1957). The results for all the parameters were subjected to two-way analysis of variance, and the least significant differences (LSD) were calculated following Snedecor and Cochran (1980) for comparing means.

Results and discussion

The increasing external Na/Ca ratios had an inhibitory effect on the growth of *C. pennisetiformis* and *P. turgidum,* whereas that of *L. fusca* remained unaffected. Number of tillers per plant and number of green leaves per tiller (Table 2) decreased markedly in *C. pennisetiformis* at the highest Na/Ca ratio, whereas these variables remained unaffected in *L. fusca* and *P. turgidum* at varying external Na/Ca ratios. The results for *C. pennisetiformis* and *P. turgidum* are in conformity with the early findings of Epstein (1961), Rains (1972), Greenway and Munns (1980), and Kent and Läuchli (1985) who reported that adequate levels of calcium may alleviate the deleterious effects of salinity on plant growth. By contrast, no effect of varying Na/Ca ratios on *L. fusca* can be explained to its occurring on highly saline-sodic soils (Malik *et al.* 1986). These types of results have been found by Yeo and Flowers (1985) who also did not find any effect of varying Na/Ca ratios in culture solution on the growth of rice.

Leaf water potential (Table 3) of *C. pennisetiformis* decreased consistently at all Na/Ca ratios, whereas that of *P. turgidum* remained unaffected. Leaf water potential of *L. fusca* decreased at an Na/Ca ratio of 99 and increased at the highest Na/Ca ratio. The results clearly demonstrate that all species and in particular *L. fusca* maintained pressure potential and so they are able to maintain growth under high salt concentration of the growth medium having low calcium concentration. In *L. fusca* it is possible to say that osmotic adjustment occurred.

Leaf diffusive resistance of *P. turgidum* increased significantly at Na/Ca ratios of 49 and 99, whereas that of the other two species remained unaffected.

The mean data for chlorophyll contents (Table 4) show that chlorophyll *a* decreased significantly ($P < 0.05$) in *L. fusca* at the highest Na/Ca ratio, whereas there was no consistent pattern of increase or decrease in chlorophyll *a* in the other two species. Chlorophyll *b* and total chlorophyll decreased consistently in *P. turgidum* with the increase in external Na/Ca ratios, whereas those of *L. fusca* decreased only at the highest Na/Ca ratio. By contrast, chlorophyll *b* and total chlorophyll in *C. pennisetiformis* remained unaffected with increasing Na/Ca

Table 2. Mean plant dry matter [g plant^{-1}], number of tillers per plant, number of green leaves per tiller of three grass species grown under variyng Na/Ca ratios for 7 weeks.

Treatment	Plant dry matter			Number of tillers/plant			Number of green leaves/tiller		
	C. penniset.	*L. fusca*	*P. turgidum*	*C. penniset.*	*L. fusca*	*P. turgidum*	*C. penniset.*	*L. fusca*	*P. turgidum*
T_0	125.5	92.2	97.0	26.3	22.0	16.0	4.0	4.8	5.0
T_1	72.9 a*	73.6 a	73.5 a	20.0 a	17.0 a	15.0 a	4.0	4.0	5.0
	x**	x	x	x	xy	y			
T_2	51.8 b	60.0 b	67.2 a	15.3 b	16.6 a	13.3 a	3.3	4.2	3.0
	x	xy	y	x	x	x			
T_3	58.0 b	77.4 a	52.1 b	21.0 a	16.0 a	13.0 a	4.0	4.0	4.3
	x	y	x	x	y	y			
T_4	57.8 b	73.9 a	39.4 c	14.0 b	19.0 a	16.0 a	2.0	4.0	4.0
	x	y	z	x	y	xy	LSD 5% = NS		

Means with the same letters in each column* and each row** do not differ significantly at 5 % level

Table 3. Mean leaf water potential (ψ_W), osmotic potential (ψ_s), turgor potential (ψ_p), and leaf diffusive resistance of three grass species grown at various Na/Ca ratios for 7 weeks. *(C. penn. - C. pennisetiformis, P. tur. - P. turgidum)*

Treatment	ψ_w [- MPa]			ψ_s [- MPa]			ψ_p [MPa]			Leaf resistance [s cm^{-1}]		
	C. penn.	*L. fusca*	*P. tur.*	*C. penn.*	*L.fusca*	*P. tur.*	*C. penn.*	*L. fusca*	*P. tur.*	*C. penn.*	*L. fusca*	*P. tur.*
T_0	1.75	2.05	1.60	1.53	1.27	1.09	0.20	0.78	0.51	5.38	4.16	6.30
T_1	1.80 a*	2.40 a	2.10 a	1.93 a	1.86 a	1.63 a	0.43 a	0.55 ab	0.47 ab	4.67 a	5.19 a	4.96 a
	x**	y	x	x	x	x	x	x	x	x	x	x
T_2	2.15 b	2.50 a	1.90 a	1.84 a	2.05 a	1.49 a	0.31 a	0.45 a	0.41 a	4.50 a	4.72 a	5.67 b
	x	y	x	xy	x	y	x	x	x	x	x	y
T_3	2.35 b	3.00 b	1.80 a	1.30 b	2.33 b	1.85 a	0.31 a	0.69 b	0.30 a	5.14 a	5.00 a	5.69 b
	x	y	z	x	y	z	x –	y	x	yx	x	y
T_4	2.45 b	1.85 c	1.86 a	1.78 a	2.50 b	1.50 a	0.68 b	0.65 b	0.70 b	4.73 a	5.00 a	5.00 a
	x	y	y	x	y	x	x	x	x	x	x	x

Means with the same letters in each column* and each row** do not differ significantly at 5 % level.

Table 4. Mean chlorophyll *a*, chlorophyll *b* and total chlorophyll content [mg g^{-1} (fresh tissue)] of three grass species grown under varying Na/Ca ratios for 7 weeks.

Treatment	Chlorophyll *a*			Chlorophyll *b*			Total chlorophyll		
	C. penniset.	*L.. fusca*	*P. turgidum*	*C. penniset.*	*L. fusca*	*P. turgidum*	*C. penniset.*	*L. fusca*	*P. turgidum*
T_0	1.33	1.17	0.79	0.88	0.82	0.58	2.52	2.26	1.61
T_1	1.14 ab*	1.38 a	1.27 a	0.77 a	1.13 a	0.79 a	2.04 ab	3.17 a	2.57 a
	x**	x	x	x	y	x	x	y	z
T_2	1.40 ac	1.35 a	0.94 b	0.79 a	1.71 b	0.54 b	2.48 ac	3.75 b	1.78 b
	x	x	y	x	y	x	x	y	z
T_3	1.04 b	1.40 a	1.04 ab	0.74 a	1.34 a	0.46 b_	1.87 b	3.48 ab	1.98 b
	x	y	x	x	y	z	x	y	x
T_4	1.64 c	1.00 b	0.99 ab	0.86 a	0.68 c	0.49 b	2.61 c	2.09 c	1.83 b
	x	y	y	x	xy	y	x	xy	y

Means with the same letters in each column* and each row** do not differ significantly at 5 % level.

Table 5. Mean content of leaf soluble proteins, leaf free amino acids, leaf proline, and total sugars of both shoots and roots of three grass species grown under varying Na/Ca ratios for 7 weeks *(C. pen. - C. pennisetiformis, P. tur. - P. turgidum, L. fus. - L. fusca)*

Treatment	Soluble proteins [mg g^{-1} (fresh mass)]			Free amino acids [μg g^{-1} (fresh mass)]			Proline [μmol g^{-1} (fresh mass)]			Sugar [mg g^{-1} (dry mass)] shoots			roots		
	C. pen.	*L. fus.*	*P. tur.*	*C. pen.*	*L. fus.*	*P. tur.*	*C. pen.*	*L. fus.*	*P. tur.*	*C. pen.*	*L. fus.*	*P. tur.*	*C. pen.*	*L. fus.*	*P. tur.*
T_0	0.60	1.20	1.15	64.4	78.1	98.8	1.32	1.32	3.78	104.7	96.3	109.5	85.5	60.2	78.0
T_1	1.01	1.20	1.25	104.4a*	92.5a	141.3a	0.83a	3.12 a	2.87 a	106.1	97.1	95.9	116.2	63.3	77.7
				x**	x	y	x	y	y						
T_2	0.85	1.05	1.15	105.6a	94.4a	116.3b	0.90a	2.12ab	3.16a	108.9	81.4	115.2	77.7	74.0	78.7
				xy	x	y	x	y	y						
T_3	0.87	1.30	1.10	76.9b	66.3b	132.5ac	0.62a	2.15a	2.36a	97.7	95.9	110.1	112.7	78.1	68.6
				x	x	y	x	y	y						
T_4	0.85	1.15	1.16	106.9a	68.1b	118.1bc	1.20a	1.00b	2.70a	116.9	98.0	100.7	93.4	68.3	72.7
				x	y	x	x	x	y						
	LSD (Spp.XT) = NS									LSD (Spp.XT) = NS for shoots and roots					

Means with the same letters in each column* and each row** do not differ significantly at 5% level.

ratios in the growth medium.

The leaf free amino acids (Table 5) decreased significantly ($P < 0.05$) at the two higher Na/Ca ratios, whereas those of *P. turgidum* decreased at Na/Ca ratios of 49 and 199 and those of *C. penniseliformis* only at an Na/Ca ratio of 99.

The leaf proline content (Table 5) of *L. fusca* decreased significantly at the highest external Na/Ca ratio but that of the other two species remained unaffected. Accumulating proline has been often found in plants subject to salt stress (Rains 1981, Wyn Jones 1980). But the proline content of measured species remained unchanged expect *L. fusca* in which the proline content decreased with the increase in Na/Ca ratios of the growth medium. These results are in conformity with the early findings of Moftah and Michel (1987) and Ashraf (1989) who could not find any difference in proline content of some tolerant and sensitive cultivars of *Glycine max* and *Vigna mungo*, respectively.

Although there was a considerable difference in growth of the three species in response to varying Na/Ca ratios of the growth medium, correlations between growth and different physiological and biochemical variables determined in this study are not possible to be established.

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