Alleviation of sodium chloride induced inhibition of growth and nitrogen metabolism of clusterbean by calcium

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Abstract

Increasing NaCl concentration (0, 50, 100 and 150 mM) progressively decreased growth and seed yield of clusterbean (*Cyamopsis tetragonoloba* Taub.) which was associated with decreased concentrations of potassium and calcium and increased concentration of sodium in the shoots. Supplemental calcium (2.5 and 5.0 mM) significantly ameliorated the adverse effects of NaCl due to enhanced Ca and K uptake and reduced Na uptake. Calcium also alleviated the negative effects of NaCl on activities of nitrogen metabolism enzymes as well as on contents of soluble protein and free amino acids.

Additional key words: *Cyamopsis tetragonoloba*, ion uptake, proteins.

Introduction

The adverse effects of salinity on plants have been attributed to low external water potentials, specific ion toxicity and ion imbalance. The degree to which each of these factors affects growth depends on the plant species, environmental conditions and salinity levels. Calcium can reduce the adverse effects of NaCl salinity in a number of crops (LaHaye and Epstein 1971, Hanson 1984, Cramer *et al.* 1989, Akhavan-Kharazian *et al.* 1991). Elevated Ca levels may protect the plants from NaCl toxicity by reducing displacement of membrane-associated calcium (Cramer *et al.* 1985, Lynch and Läuchli 1988) and/or by reducing sodium uptake and its transport to the shoots (LaHaye and Epstein 1971, Cramer *et al.* 1987, Rengel 1992, Gorham and Bridges 1995). Calcium also improves potassium uptake under NaCl salinity (Cramer *et al.* 1985, Cheeseman 1988, Cachorro *et al.* 1994) thereby effectively increasing the K/Na ratio in the tissues. However, the ameliorative role of Ca on nitrogen metabolism has not been sufficiently elucidated (Ward *et al.* 1986, Pessarakli *et al.* 1989, Garg *et al.* 1993). Therefore, the objective of the present study was to explore the role of Ca in ameliorating the NaCl effects on growth, ion concentration and

Received 15 March 1996, *accepted* 15 January 1997.
Acknowledgement: Thanks are due to the Director, Central Arid Zone Research Institute, Jodhpur for providing the facilities. Technical help rendered by Mr. M.R. Naval and A.R. Sudi is duly acknowledged.

nitrogen metabolism of clusterbean, an important grain and fodder legume, which is widely grown in salt affected dry lands of western Rajasthan in India.

Materials and methods

Clusterbean (*Cyamopsis tetragonoloba* Taub cv. HFG-182) plants were grown in glazed pots (2 plants/pot) having 40 kg loamy sand soil containing 7.1, 5.9, 63.1 and 24.1 % of clay, silt, fine sand and coarse sand, respectively, and having 0.15 % organic carbon and 0.023 % total nitrogen. The available nitrogen, phosphorus and potassium corresponded to 80, 12 and 120 kg ha^{-1}, respectively. Twenty days after sowing pots were salinized by solution of 0, 50, 100 and 150 mM NaCl in combination with $CaCl_2$ (0, 2.5 and 5.0 mM). The plants were irrigated with appropriate NaCl and/or $CaCl_2$ solutions on 20, 25 and 30 d after sowing (DAS) by providing 2 dm^3 solution to each pot every time. The control plants received only tap water. Soil moisture was maintained close to field capacity by frequent watering with tap water (0.2 dS m^{-1}) after the imposition of salinity treatments. The 12 treatments had 15 replications each.

At the flowering stage (50 DAS) the two upper (fully expanded) leaves (from 6 plants for each treatment) were analyzed for soluble proteins (Lowry *et al.* 1951), free amino acids (Yemm and Cocking 1955) and the activities of nitrate reductase (Jaworski 1971), glutamine synthetase (Elliott 1953), glutamate synthase (Boland *et al.* 1978) and glutamate dehydrogenase (Boland *et al.* 1978). Plant growth was determined from leaf area (using *LiCor LI-3000* leaf area meter) at the flowering stage, final plant height and final dry matter of shoots, pods and seeds. Samples of shoot dry mass were analyzed in triplicate for Na, K and Ca (Jackson 1973) contents at the flowering stage and at harvest. The statistical significance of the differences was ascertained by analysis of variance.

Results and discussion

Increasing NaCl salinity progressively decreased shoot, pod and seed dry mass (Table 1). $CaCl_2$ application partly alleviated the adverse effects of NaCl. Consequently the reduction in growth and yield at all concentrations of NaCl was less in presence of calcium. NaCl decreased plant height and leaf area at the flowering stage (Table 1). In the case of leaf area the effect was significant even at 50 mM NaCl. However, plant height was significantly reduced only at 150 mM NaCl. Ca application marginally increased plant height. However, Ca application significantly overcame the adverse effects of NaCl on leaf area, and markedly increased it at all levels of salinity.

Sodium concentration in the shoot tissue progressively increased with increasing NaCl concentration both at the flowering stage and at harvest (Table 2). Addition of Ca reduced the Na concentration in the shoot tissue at all salinity levels. Increasing

Table 1. Influence of different concentrations [mM] of NaCl (0, 50, 100 and 150) and $CaCl_2$ (0, 2.5, or 5.0) on shoot, pod and seed dry mass [g $plant^{-1}$], plant height [cm] and leaf area [cm^2 $plant^{-1}$].

NaCl	Shoot mass			Pod mass			Seed mass			Plant height			Leaf area		
[mM]	0	2.5	5.0	0	2.5	5.0	0	2.5	5.0	0	2.5	5.0	0	2.5	5.0
0	33.4	33.8	33.8	21.5	22.2	22.3	11.4	11.5	11.7	125.8	125.4	124.0	1417	1477	1456
50	29.3	31.7	32.1	19.7	21.6	21.9	10.1	10.8	11.0	123.6	127.7	126.2	1347	1429	1375
100	27.1	29.6	30.1	18.4	20.5	20.8	8.6	9.9	10.1	116.1	118.6	120.6	1142	1202	1225
150	24.0	26.1	27.5	16.9	19.0	19.7	7.6	9.0	9.1	110.0	110.3	114.3	1070	1115	1166
LSD at P =	0.05	0.01		0.05	0.01		0.05	0.01		0.05	0.01		0.05	0.01	
NaCl	1.40	1.90		0.90	1.30		0.40	0.60		12.50	16.10		57.00	77.00	
$CaCl_2$	1.30	2.00		1.00	1.50		0.50	0.70		NS	NS		64.00	93.00	
Na × Ca	2.50	3.40		1.70	2.40		0.80	1.20		NS	NS		107.00	148.00	

Table 2. Influence of different levels of NaCl (0, 50, 100, 150 mM) and $CaCl_2$ (0, 2.5, 5.0 mM) on concentrations of Na, K and Ca and K/Na ratios in the shoot tissue of clusterbean at flowering and harvest stages.

NaCl [mM]	$CaCl_2$ [mM] flowering 0	2.5	5.0	harvest 0	2.5	5.0
Sodium [μmol g^{-1}(d.m.)]						
0	100	70	66	83	66	61
50	223	166	157	127	125	96
100	302	236	223	153	127	114
150	333	263	240	226	166	153
LSD at	NaCl	$CaCl_2$	Na × Ca	NaCl	$CaCl_2$	Na × Ca
$P = 0.05$	16	9	25	9	6	15
Potassium [μmol g^{-1}(d.m.)]						
0	716	752	760	519	549	535
50	683	737	749	509	532	532
100	639	703	703	476	499	509
150	619	682	693	470	486	494
LSD at	NaCl	$CaCl_2$	Na × Ca	NaCl	$CaCl_2$	Na × Ca
$P = 0.05$	47	27	75	29	12	54
Calcium [μmol g^{-1}(d.m.)]						
0	116	168	191	92	137	169
50	110	151	185	91	130	169
100	101	142	170	78	123	151
150	87	128	153	73	118	139
LSD at	NaCl	$CaCl_2$	Na × Ca	NaCl	$CaCl_2$	Na × Ca
$P = 0.05$	10	9	NS	8	7	NS
K/Na ratio						
0	7.12	10.73	11.56	6.24	8.36	8.72
50	3.06	4.43	4.76	4.01	4.25	5.52
100	2.12	2.98	3.15	3.11	3.93	4.47
150	1.86	2.60	2.78	2.08	2.92	3.22

NaCl decreased the tissue concentration of K whereas the presence of Ca overcame this effect and tended to enhance the K intake at all NaCl concentrations (Table 2). The positive influence of Ca addition was more pronounced at the flowering stage than at harvest. Thus Ca application increased the K/Na ratio at all levels of NaCl. Increasing NaCl concentrations also decreased the Ca content of plants at the flowering stage as well as at harvest. Addition of Ca significantly increased Ca concentration of control and NaCl stressed plants. Although the increase in Ca concentration was reduced by increasing levels of NaCl, but Ca content, as such, remained much higher in plants supplied with Ca compared to those plants where Ca was not applied. The results obtained with clusterbean agree with the results obtained generally with other crops (Cramer *et al.* 1987, Hansen and Munns 1988, Akhavan-Kharazian *et al.* 1991, Cramer *et al.* 1991, Rengel 1992, Bernstein *et al.* 1993, Gorham and Bridges 1995).

Table 3. Influence of different levels of NaCl (0, 50, 100, 150 mM) and $CaCl_2$ (0, 2.5, 5.0 mM) on soluble protein, free amino acids and the activities of NR, GS, GOGAT and GDH in leaves of clusterbean at the flowering stage.

NaCl [mM]	$CaCl_2$ [mM] 0	2.5	5.0	LSD at	$P = 0.05$	$P = 0.01$
Soluble protein [mg g^{-1}(d.m.)]						
0	65.3	70.5	65.5	NaCl	4.9	6.7
50	52.1	76.6	82.0	$CaCl_2$	3.9	6.5
100	42.8	51.2	60.8	Na × Ca	8.3	11.8
150	36.0	43.1	50.0			
Free amino acids [mg g^{-1}(d.m.)]						
0	13.2	14.8	12.9	NaCl	1.2	1.7
50	16.2	17.7	16.9	$CaCl_2$	1.2	2.0
100	11.4	13.6	12.9	Na × Ca	2.2	3.1
150	9.7	12.7	10.8			
Nitrate reductase [μg(NO_2) g^{-1}(d.m.) s^{-1}]						
0	0.048	0.057	0.066	NaCl	0.003	0.004
50	0.028	0.034	0.039	$CaCl_2$	0.003	0.005
100	0.022	0.033	0.036	Na × Ca	0.006	0.008
150	0.019	0.029	0.032			
Glutamine synthetase [ΔA_{540} g^{-1}(d.m.) s^{-1}]						
0	0.084	0.082	0.083	NaCl	0.006	0.009
50	0.079	0.075	0.077	$CaCl_2$	0.005	0.008
100	0.061	0.069	0.072	Na × Ca	0.011	0.015
150	0.044	0.051	0.053			
Glutamate synthase [ΔA_{340} g^{-1}(d.m.) s^{-1}]						
0	0.023	0.023	0.024	NaCl	0.002	0.003
50	0.019	0.021	0.021	$CaCl_2$	0.002	0.003
100	0.017	0.020	0.020	Na × Ca	0.003	0.005
150	0.014	0.017	0.019			
Glutamate dehydrogenase [ΔA_{340} g^{-1}(d.m.) s^{-1}]						
0	0.016	0.016	0.015	NaCl	0.001	0.001
50	0.014	0.018	0.022	$CaCl_2$	0.001	0.001
100	0.011	0.014	0.015	Na × Ca	0.002	0.003
150	0.006	0.008	0.026			

Favourable effects of Ca application were also apparent on leaf metabolites (Table 3). the NaCl induced decrease in soluble protein content was greatly overcome by addition of Ca, particularly at 5.0 mM Ca concentration. Likewise levels of free amino acids decreased with increasing concentrations of NaCl beyond 50 mM while Ca application tended to increase these levels at all concentrations of NaCl. The adverse effects of salinity on protein and amino acids are well known, but the reversal of these effects by Ca has not been sufficiently elucidated. The results indicate that presence of Ca under salinity is conducive to a more efficient leaf nitrogen metabolism.

This contention is further supported by changes in the activities of certain leaf enzymes of nitrogen and ammonia assimilation (Table 3). Nitrate reductase (NR), glutamine synthetase (GS), glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH) activities progressively and significantly declined with increasing NaCl concentration. Ca application significantly arrested these declines and markedly increased their activities.

It is noteworthy that Ca addition was able to restore the GDH activity to almost control values upto 100 mM level of NaCl salinity. But at 150 mM NaCl concentration there was only marginal improvement by Ca application.

The inhibitory effects of salt stress on the activities of aforesaid enzymes have been reported by several workers in a number of crops including clusterbean (Garg *et al.* 1986, Lahiri *et al.* 1987). However, Ca mediated alleviation of salinity effects on leaf enzymes has not received attention in these studies. Ward *et al.* (1986) found that increasing Ca concentration in saline nutrient solution resulted in increase in nitrate assimilation and growth of barley seedlings. The enhancement of nitrate transport by Ca under saline conditions was dependent on the presence of Ca in the uptake solution along with the salt.

Data presented indicate that favourable effects of Ca application under NaCl salinity are not only at the level of nutrient uptake and plant growth, but they also favourably influence the plant metabolism and thereby protect the tissues from toxic effects of salinity at the sub-cellular level.

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