Osmoregulation in Higher Plants: Effect of Maintaining a Constant Na : Ca Ratio on the Growth, Ion Balance and Organic Solute Status of NaCl Stressed Cowpea (*Vigna sinensis* L.)

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Summary

Plants of *V. sinensis* tolerated NaCl concentrations as high as 150 mM in their growth media and showed a tendency to halophility by exhibiting a higher yield up to 50 mM $NaCl_{ext}$. A constant Na/Ca ratio of 5.0 with increasing $NaCl_{ext}$ helped to alleviate the adverse effect of high salinity. This was evidenced by an improvement in all the growth parameters. The presence of higher Ca^{+2} helped to prevent passive entry of Na^+ and in no case did the endogenous K/Na ratio fall below 1.0. The status of all the other cations and anions was also modified. In contrast, the ratio of endogenous K/Na was lower than 1.0 when Na/Ca_{ext} was not held constant with increasing $NaCl_{ext}$ concentration. In both situations Cl^- accumulated to a very high extent. The accumulation of some organic solutes related to stress resistance and/or metabolism was different under the two different conditions of culture. Measurement of RWC showed that a water deficit induced by salinity was not the principal cause of growth reduction under salt stress.

Of the inorganic ions, K^+ and NO_3^- played the key role in osmoregulation. However, at high salinities the function of Na^+, tolerated to some extent by the plants, must also be considered. The amount of NO_3^- accumulated indicated that this plant is probably a nitrophile.

Among the organic solutes, soluble sugars and polyols responded positively to salinity indicating their role as the principal organic osmotica. Free proline and quarternary ammonium compounds behaved as stress indicators.

Key words: Vigna unguiculata syn sinensis, constant Na : Ca ratio, ion excess, osmoregulation, polyols, proline, quarternary ammonium compounds, salt stress.

Introduction

The presence of NaCl in the growth media of leguminous plants has been found to have different effects on their growth and solute status even though the plants adapt to the adverse condition (Imamul Huq and Larher, 1983 a, 1983 b; Nukaya et al., 1982 a, 1982 b; Winter and Läuchli, 1982). The presence of high Na in the ex-

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Abbreviations: $NaCl_{ext}$ = NaCl solution applied to the growth medium; $CaCl_{2ext}$ = $CaCl_2$ solution applied to the growth medium; O. M. = organic matter; QAC = quarternary ammonium compounds; RWC = relative water content.

ternal media lowers the Na/Ca ratio and such a situation gives rise to a passive accumulation of Na ultimately causing an «ion excess» effect (Greenway and Munns, 1980). This is thought to be the primary reason for growth reduction and changes in the chemical composition of higher glycophytes under saline conditions. Ca^{+2} concentrations between 1 and 5 mM are generally required to protect the roots of plants from the deleterious effects of, among other things, salinity and ion imbalance (Clarkson and Hanson, 1980). By serving to ward off membrane damage and leakiness Ca^{+2} plays a key role in the selective transport of K^+ – the principal osmoticum in glycophytes – in the presence of excess Na^+, thereby making the plants more salt tolerant (Clarkson and Hanson, 1980; Epstein, 1980). Insufficient information is available on the influence of Ca^{+2} in ameliorating the toxic effect of high $NaCl_{ext}$ on leguminous plants. The influence of different concentrations of Ca^{+2} at a constant $NaCl_{ext}$ on the bean have been reported (Epstein, 1980; Greenway and Munns, 1980). Rayer (1981) showed that Ca^{+2} exerted a stimulatory effect on the growth of soybean. Raymond et al. (1965) observed a depressive effect of Ca^{+2} on the non-metabolic uptake of Na^+ in maize roots. However, the influence of Ca^{+2} on the growth and subsequently on the composition of leguminous plants by keeping a constant Na/Ca ratio with changing $NaCl_{ext}$ concentrations, has so far not been studied. The present work was undertaken to study the effect of a constant Na : Ca ratio with varying $NaCl_{ext}$ concentrations (from 10 to 150 mM) on the growth, ion balance and some organic solutes of a moderately salt tolerant leguminous plant (Imamul Huq and Larher, 1983 c), *V. sinensis*, commonly known as cowpea, as compared to plants in which the Na : Ca was not kept constant.

Materials and Methods

Plant material and culture

Seeds of cowpea (*Vigna unguiculata* (L.) Walp. Syn. *V. sinensis* (L.) Savi ex Hassk.) were germinated directly on vermiculite and allowed to grow under controlled conditions with mineral N in the presence of either 0, 10, 25, 50, 100 and 150 mM NaCl ($NaCl_{ext}$) or the same concentrations of $NaCl_{ext}$ plus 2, 2, 5, 10, 20 and 30 mM $CaCl_2$ ($CaCl_{2ext}$) to maintain a constant Na : Ca ratio of 5.0, except for the control. The source of the seeds, composition of the nutrient solution and growing conditions are the same as described elsewhere (Imamul Huq and Larher, 1983 a, 1983 c). Plants were always harvested 35 days after the emergence of seedlings. The treatments with $NaCl_{ext}$ are designated as C_1, C_2, C_3, C_4, C_5, and C_6, respectively, for the different salt concentrations, and C'_1, C'_2, C'_3, C'_4, C'_5 and C'_6 for the treatments corresponding to the different $CaCl_{2ext}$ concentrations.

Both nutrient solutions and the salts were renewed weekly. Distilled water was added daily to the vermiculite to maintain a constant moisture content in the growing medium. At harvest, the root free space was unloaded by three 1 min washings in 1 l distilled water. Lengths and fresh weights of shoots and roots were measured. Dry matter was determined by drying at 85 °C for 48 h. Relative water content (RWC) of the leaves at the time of harvest was measured following the method of Weatherly (1950). Organic matter (O. M.) was estimated by deducting total quantified mineral matter from total dry weight.

Analyses

Inorganic ions: Na^+ and K^+ were determined by flame photometry and Ca^{+2} and Mg^{+2} by atomic absorption spectrophotometry following the same techniques as described in Imamul Huq and Larher (1983 a). NO_3^- and Cl^- were extracted from the dry material in water at 45 °C for 1 h and P was extracted as total P by mineralizing the dry material in a ternary acid mixture. The methods of determination of these compounds have been described in Imamul Huq and Larher (1983 a).

Organic solutes: The extraction of soluble organic solutes from the dried plant materials and the subsequent determination of free amides, free amino acids, free proline and quarternary ammonium compounds have been described in Imamul Huq and Larher (1983 b). Free sugars was determined using anthrone reagent (IRRI, 1976) and total polyols by the method described by Vinning and Taber (1974). Free sugars are expressed as equivalents of glucose and total polyols as equivalents of sorbitol.

Results

Growth

Growth in the presence of increasing $NaCl_{ext}$

The seeds of cowpea readily germinated in the presence of NaCl concentrations as high as 150 mM. However, at 150 mM of salt the process was delayed. Although vegetative development was normal in all the salt treatments, it seemed better in the presence of 10–25 mM $NaCl_{ext}$ than in the control. Above 50 mM NaCl the growth rate appeared to be retarded, which was evident from the fact that tendrils did not appear in the plants treated with 100 and 150 mM NaCl even at harvest.

The heights of the salt treated plants were less than those in the controls except for the roots of C_2 plants (Figures 1 a and 1 b). That growth in the presence of a low concentration of $NaCl_{ext}$ was better than in the controls was appreciable from the increase in both fresh and dry weights of the shoots and roots up to 50 mM NaCl (Figs. 1 a and 1 b). The decrease in root dry weight at 100 mM NaCl was only 1 % that of the control. Production of O. M. decreased to about 55 % at C_6. The decrease in all the growth parameters at higher salinity was significantly correlated with external salinity («r» significant at 1 and 5 %).

Growth in the presence of increasing $NaCl_{ext}$ at a constant Na : Ca ratio of 5.0

The seeds germinated in the presence of the whole range of salt concentrations although the rate of germination and subsequent plant development were slower in the presence of 150 mM NaCl + 30 mM $CaCl_2$. Increasing the $CaCl_{2ext}$ concentration with a concomitant increase in $NaCl_{ext}$ concentration helped to overcome the toxic effect of salinity. All the growth parameters measured showed a substantial improvement (Figs. 1 c and 1 d). Although the plants are usually shorter in the presence of $NaCl_{ext}$ (Imamul Huq and Larher, 1983 a; 1983 c; Nieman, 1965), they were longer than the control in the presence of salt. The changes in the length of both the plant parts were greater or equal to those in the controls up to C'_5. Neither the fresh

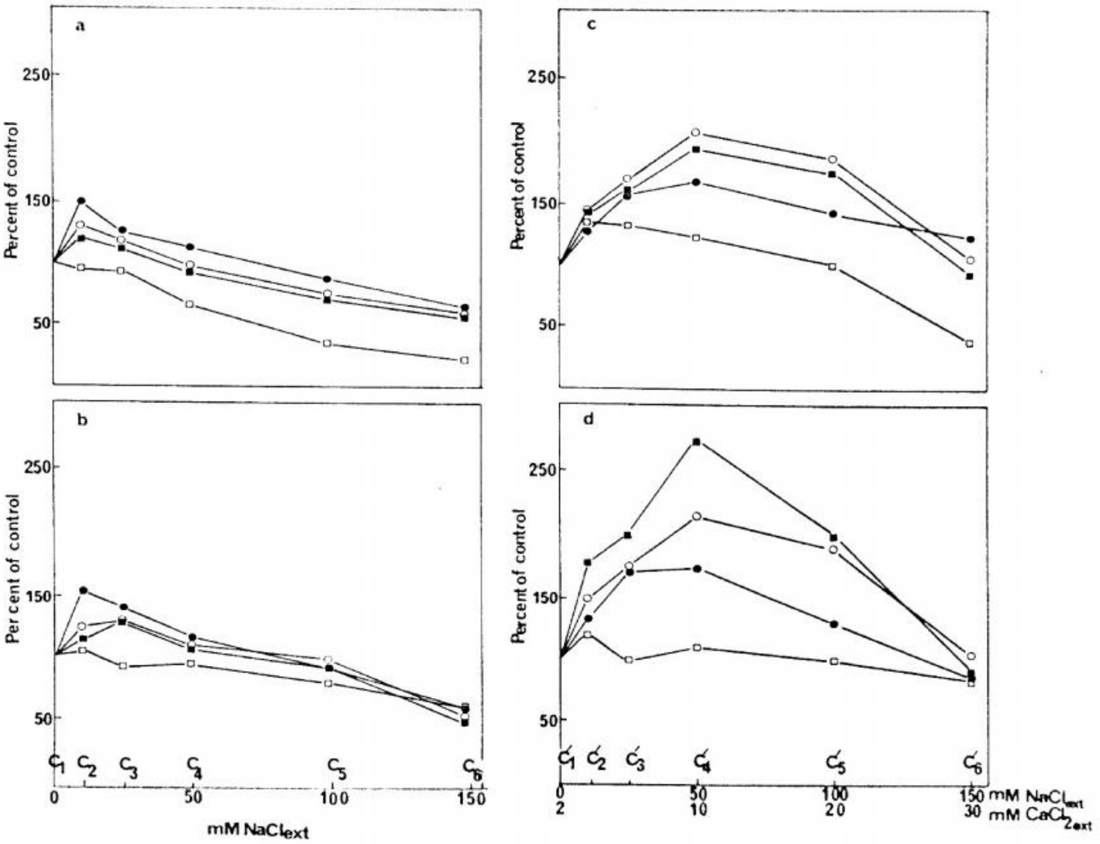

Fig. 1: Various growth parameters of cowpea as affected by increasing NaCl$_{ext}$ concentration, (a) shoot, (b) root and by increasing NaCl$_{ext}$ when the Na/Ca$_{ext}$ was kept constant, (c) shoot, (d) root. C_1, C_2, C_3, C_4, C_5, C_6 and C'_1, C'_2, C'_3, C'_4, C'_5 and C'_6 represent respectively NaCl$_{ext}$ concentrations of 0, 10, 25, 50, 100 and 150 mM and the same concentrations of NaCl plus 2, 2, 5, 10, 20 and 30 mM CaCl$_{2ext}$. (□) length; (●) fresh weight; (○) dry weights; (■) organic matter.

nor the dry weight of shoots showed a decrease in the presence of the whole range of salt concentrations. The maximum increase in shoot weight was observed with the combination of 50 mM NaCl + 10 mM CaCl$_2$, i. e., 142 % of the control for fresh weight and 207 % for dry weight. The same was also true for the root weights. Organic matter production was also improved when the Ca^{+2} concentrations was kept high in the presence of increasing NaCl$_{ext}$ concentration. The amount of organic matter increased with increasing NaCl$_{ext}$ up to 100 mM where it was 200 % of the control for the roots. The maximum O. M. production was obtained with the same combination of NaCl and CaCl$_2$, the plant heights also being maximum. The O. M. production was 195 % of the control for the shoot and 273 % for the root. The de-

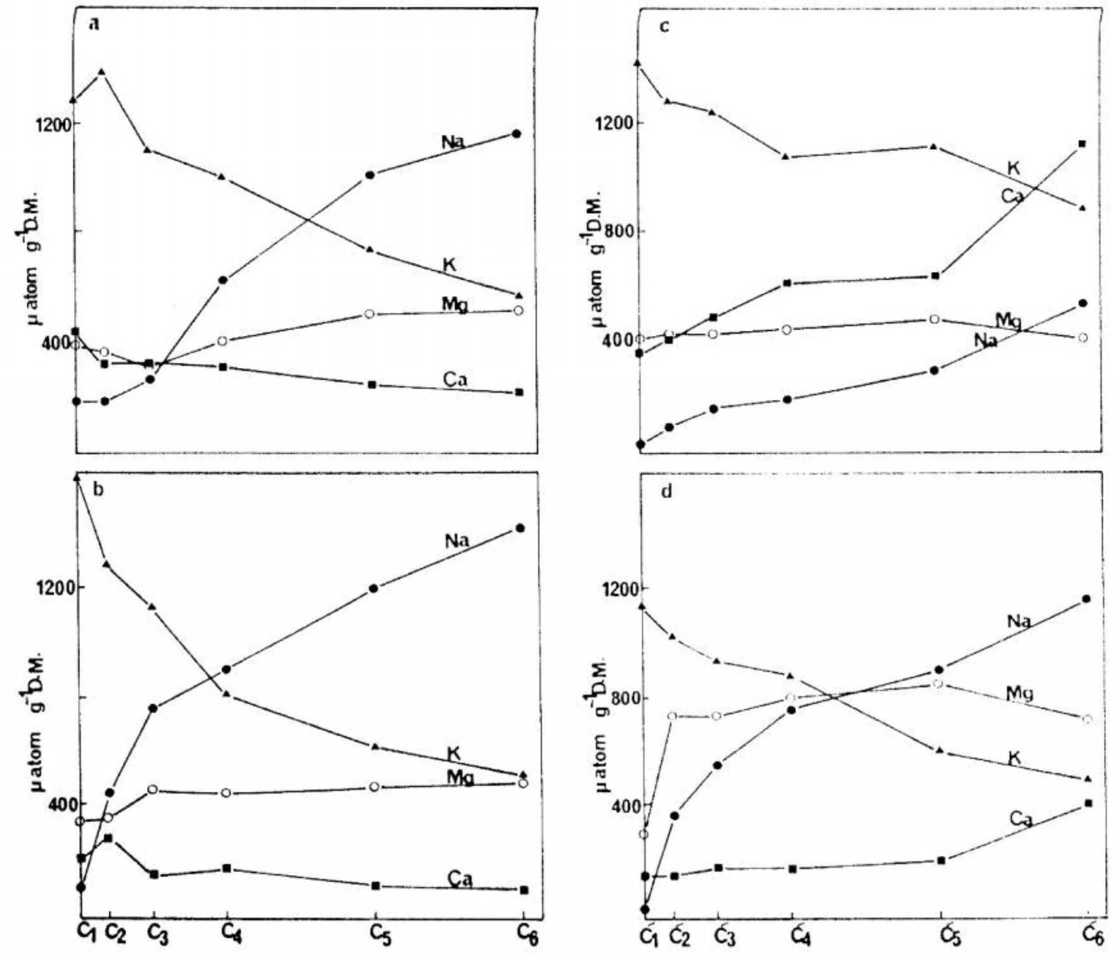

Fig. 2: Cation balance in cowpea as affected by increasing $NaCl_{ext}$ concentrations, (a) shoot, (b) root and by increasing $NaCl_{ext}$ when Na/Ca_{ext} was kept constant, (c) shoot, (b) root. $C_1 \ldots C_6$ and $C'_1 \ldots C'_6$ represent the treatments as indicated in Fig. 1.

crease at C'_6 was only 7 % for the shoot and 9 % for the root. The correlation coefficient values calculated between the various growth parameters and external salt concentrations were very weakly related.

Ion Balance: Results concerning the ion balance in different plant parts under the two different conditions are presented in figures 2 (a, b, c, d) for cations and 3 (a, b) for anions.

Cations in plants treated with increasing $NaCl_{ext}$ concentration

Na^+ in both shoots and roots increased significantly with increasing $NaCl_{ext}$ («r» = 0.9766*** for shoot and 0.9347** for root) whilst K^+ decreased («r» = –0.9636*** for shoot and –0.9221** for root) except at C_2 where the shoot K^+ content was about 9 % higher than in the control. It is interesting to note that the Na^+ content in the shoot at this concentration of $NaCl_{ext}$ was the same as that of the control (Figs. 2 a, 2 b). However, the plant tissues maintained substantial amounts of K^+ along with Na^+ within the $NaCl_{ext}$ ranges up to 50 mM. This was apprehended via the endogenous K : Na ratio which decreased to less than 1.0 only in the shoots at C_5 and in the roots at C_4. Mg^{+2} accumulated in both the organs with increasing salinity with an initial decrease up to 25 mM NaCl in the shoots while Ca^{+2} decreased significantly («r» = –0.8860** for shoot and –0.7679** for root) in both. However, Ca^{+2} increased to about 38 % in the roots of C_2 plants, which might explain the presence of relatively less Na^+ in and better growth of the plants under this treatment. The decrease at C_6 of the Ca^{+2} content was about 48 % in the roots and 50 % in the shoots.

Cations in plants treated with increasing $NaCl_{ext}$ at constant Na : Ca ratio

Under these conditions, the trends in Na^+ and K^+ accumulation were similar to those observed in the previous experiments. The Na^+ level increased (Figs. 2 c, 2 d) in both the organs with salinity («r» = 0.9809*** for shoots and 0.9334** for roots) while the level of K^+ decreased («r» = –0.9229** for shoots and –0.9835*** for roots). But the level of Na^+ in the roots and its subsequent transfer to the shoots was much more restricted. In no case did the endogenous K : Na ratio in shoots drop below 1.0 while in the roots it was such only at C'_5 and C'_6. The presence of Ca^{+2} modified the non-metabolic entry of Na^+ into the plants. This became more evident when the levels of endogenous Na^+ in the control plants of the two sets of experiments (C_1 and C'_1) were compared. The levels of Na^+ were 83 and 70 % less, respectively, in the shoot and root of C'_1 plants than in those of C_1, where the external Ca^{+2} concentration was 1.5 mM (that of the nutrient solution). Keeping a constant Na/Ca ratio with increasing $NaCl_{ext}$ also modified the accumulation of Ca^{+2} in plants. The ion accumulated more in both the plant parts with increasing salt concentration («r» = 0.9443** for shoots and 0.8891** for roots). Moreover, Ca^{+2} accumulation was greater in the shoots than in the roots. The Mg^{+2} status of the plants was also modified by the presence of Ca^{+2}. Mg^{+2} in the shoots did not vary much while in the roots its level increased with salinity and at C'_5 the Mg^{+2} content was about 3 fold that in the control.

Anions in plants treated with increasing $NaCl_{ext}$ concentrations

With increasing salinity, the NO_3^- content in both the plant parts showed a decrease, though not significant, with an initial increase at C_2 where the accumulation was about twice that with C_1 in both organs (Fig. 3 a). The better growth at 10 mM $NaCl_{ext}$ can presumably be related to the increase in NO_3^- uptake under this treatment. The ability of the plants to maintain appreciable concentrations of this ion (66 mM in the shoots and 35 mM in the roots at C_6) has to be emphasised since salt treated plants exhibit very poor accumulation of NO_3^-.

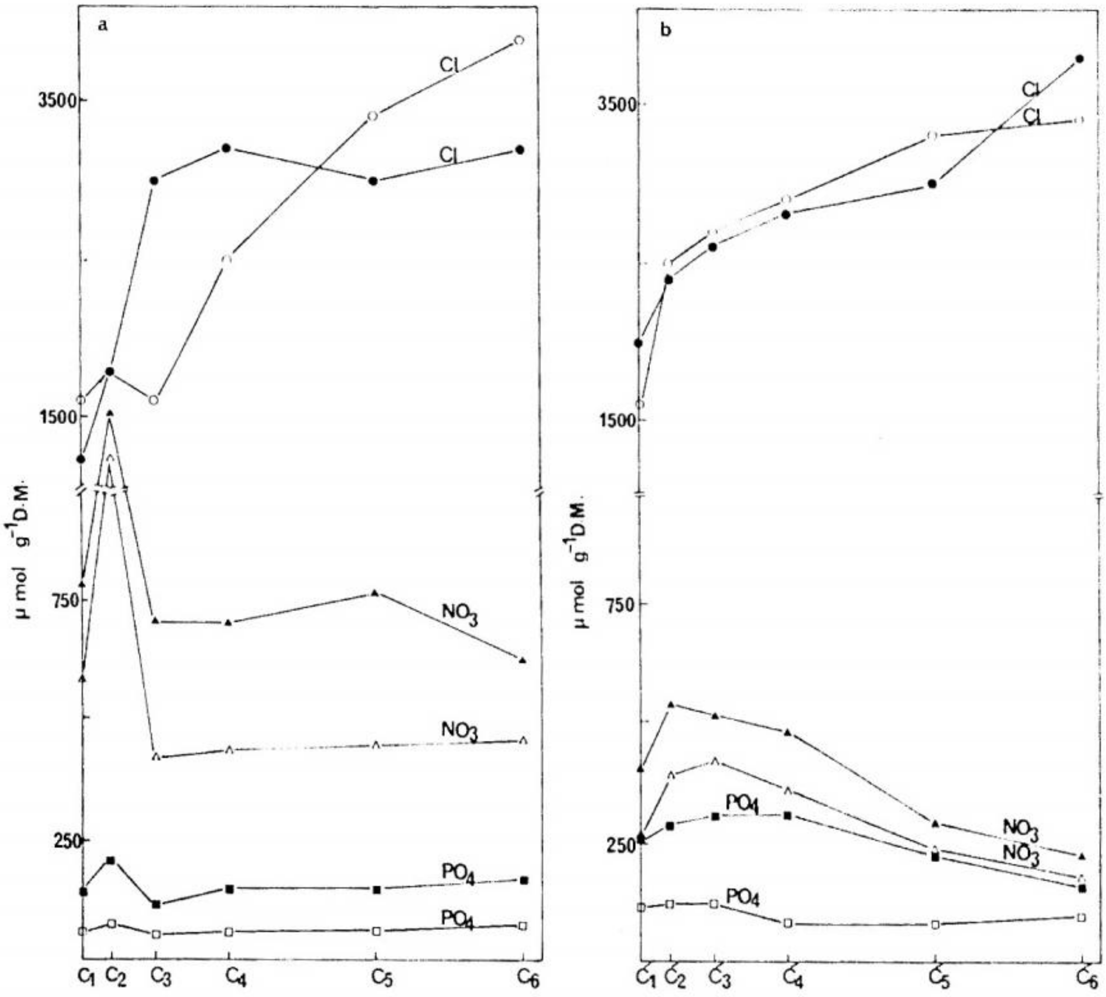

Fig. 3: Anion balance in cowpea as affected by increasing $NaCl_{ext}$ concentrations (a); and by increasing $NaCl_{ext}$ when Na/Ca_{ext} was kept constant (b). $C_1 \ldots C_6$ and $C'_1 \ldots C'_6$ represent the treatments as indicated in Fig. 1. (●) Cl^- shoot; (○) Cl^- root; (▲) NO_3^- shoot; (△) NO_3^- root; (■) PO_4^{-3} shoot; (□) PO_4^{-3} root.

Cl^- accumulation increased with increasing salinity in both shoots and roots. The accumulation of Cl^- in the roots was highly significant with external NaCl («r» = 0.9832***) while in the shoots this was not so although it was positively correlated («r» = 0.7071). It is worth mentioning here that the Cl^- concentration exceeds that required for balancing Na^+ and K^+.

Although total P does not give an actual indication of the free phosphates, yet we included the results of P in the anions to have an over all idea about the changes that undergo this element. Total P, expressed as PO_4^{-3}, increased with salinity in both the plant parts. The maximum amount was, however, observed in the plants under C_2, where the accumulation was about 1.5 times that in the control in both shoots and roots.

Anions in plants treated with increasing $NaCl_{ext}$ at constant Na : Ca ratio

A constant Na/Ca ratio in the growth media largely modified the NO_3^- accumulation in both the plant parts. The NO_3^- content in both shoots and roots was higher than in the control up to C′4. The shoots of C′2 plants accumulated about 35 % more and the roots of C′3 plants about 53 % more NO_3^- than those of control plants (C′1). The decrease at C′6 from control was about 53 and 34 %, respectively, for the shoots and roots (Fig. 3 b).

The accumulation of Cl^- did not change much although it was more significant in the shoots («r» = 0.9641**) than in the roots («r» = 0.5097) with increasing salinity. The relatively higher values for Cl^- in this set of experiments are related to the presence of a larger amount of Cl^- in the media.

The P status was also modified. At higher salt concentrations (C′5 and C′6) the total PO_4^{-3} in the shoots was lower than in the control although its accumulation increased up to C′4, where it was more than 20 % higher. In the roots, the accumulation of P was not greatly modified over the whole range of treatments.

Organic solute status

The results concerning the accumulation of various organic solutes in the plants under different salt treatments are presented in tables 1 a and 1 b.

Organic solutes in plants treated with increasing $NaCl_{ext}$ concentration (table 1 a)

At higher salinity, the amides and free amino acids increased significantly in the roots. Amides in the shoots were at a maximum in C_2 plants, i. e., 75 % more than that in C_1. The amino acid content was also at a maximum here, (859 μmol g^{-1} dry matter, i. e., 63 % more than at C_1). The lowest content of free proline was found in the C_2 plants: it increased with salinity in C_3 to C_6 plants, more so in the roots than in the shoots. The QACs expressed as glycine betaine, showed a net increase in both the plant parts, the maximum being in the C_5 shoots (ca. 44 % that of control) and in the C_6 roots (ca. 95 % that of control).

The free sugars, expressed as glucose, increased with increasing salinity both in the shoots and in the roots. However, the C_2 plants contained less sugars than the control plants (table 1 a): the maximum was in the C_6 plants which had about 48 % and 37 % more sugars than the controls respectively, in their shoots and roots. Total polyols, expressed as sorbitol, were also higher in plants treated with salt: the roots of C_6 plants accumulated about 2.5 times more than control plants. The shoots of C_2 plants accumulated more than 96 % above the control.

Organic solutes in plants treated with increasing $NaCl_{ext}$ at a constant Na : Ca ratio (table 1 b)

Contrary to the previous results, both amides and amino acids accumulated less in roots of salt treated plants than in those without $NaCl_{ext}$. The results were quite dif-

Table 1: Accumulation of various organic solutes in different plant parts of cowpea. a) Plants treated with increasing $NaCl_{ext}$; b) plants treated with increasing $NaCl_{ext}$ with the Na/Ca_{ext} kept constant. Amides are expressed as equivalent of asparagine, free amino acids as leucine, QACs as glycine betaine, free sugars as glucose and total polyols as sorbitol. The values are expressed as micromols per g dry matter.

a)

Treatment		Amides		Amino Acids		Proline		QACs		Sugars		Polyols	
mM $NaCl_{ext}$		S	R	S	R	S	R	S	R	S	R	S	R
0	C_1	244	153	527	260	13	0.5	23	18	216	158	14	7
10	C_2	427	209	859	394	0.6	0.4	20	21	187	105	26	8
25	C_3	123	97	524	334	13	4	26	17	253	158	17	5
50	C_4	293	148	547	334	15	6	20	23	286	167	19	15
100	C_5	404	289	702	561	19	9	33	24	308	194	14	10
150	C_6	236	345	575	599	15	10	31	35	320	217	15	16
Correlation coefficients «r»		0.0431	0.8485*)	−0.0155	0.8880**)	0.5506	0.9446**)	0.7680*)	0.6647	0.8874**)	0.8574*)	−0.4309	−0.0391

b)

Treatments			Amides		Amino Acids		Proline		QACs		Sugars		Polyols	
mM NaCl	mM $CaCl_2$		S	R	S	R	S	R	S	R	S	R	S	R
0	2	C'_1	161	263	253	364	0.8	21	28	24	230	156	55	15
10	2	C'_2	167	130	304	190	0.7	18	32	26	187	124	37	10
25	5	C'_3	230	138	370	167	0.5	0.2	32	26	208	102	36	8
50	10	C'_4	189	78	345	122	2	0.5	36	30	350	353	64	15
100	20	C'_5	123	71	240	99	34	3	26	41	304	138	80	23
150	30	C'_6	113	169	182	229	28	35	33	36	378	171	96	49
Correlation coefficients «r»			−0.6973	−0.3076	−0.6688	−0.3233	0.8856**)	0.4358	0.0277	0.8677*)	0.8295*)	0.2070	0.9109**)	0.9065*)

S – Shoot, R – Root; *), **) represent significance at 5 and 1 % levels respectively.

ferent in the shoots where the contents of both the solutes were higher than that at C'$_1$, the maximum being at C'$_3$. Here, the amides were about 46 % more. The proline level decreased up to C'$_3$ where it was at a minimum, afterwhich it increased with salinity. At C'$_6$, the proline content was fairly high in both the plant parts. The QACs were fairly constant in shoots and increased with increasing salinity in the roots.

The levels of free sugars in shoots increased significantly after an initial decrease up to C'$_3$. At C'$_6$, the level was more than 64 % over C'$_1$. Sugar accumulation in the roots was variable (table 1 b). A similar situation was noted with polyols which showed a net increase of about 75 % over C'$_1$ in the shoots and about 225 % in the roots at C'$_6$. A higher accumulation of compounds assumed to be stress metabolites (Hellebust, 1976; Stewart and Larher, 1980; Smith and Philips, 1982) in the C'$_1$ plants relative to those up to C'$_3$ suggests the presence of some kind of stress induced in these plants. The question arises of whether such metabolic disorders were related to an absolute requirement for Na^+ by the plants of *V. sinensis*, especially when the Ca^{+2} level was increased in the media. These phenomena were not clearly apparent in the first set of experiment.

Discussion

The present experiment was aimed at studying a) the salt tolerance of *V. sinensis* subjected to salinity stress ranging from mild to concentrations of $NaCl_{ext}$ as high as 150 mM and b) the ultimate effect of maintaining a constant Na/Ca_{ext} ratio throughout the range of NaCl concentrations used. This was evaluated by their response to germination, growth, endogenous ion balance and accumulation of some of the organic solutes assumed to be characteristic of stress.

The seeds of *V. sinensis* had little or no difficulty in germinating in the presence of high concentrations of salts (150 mM NaCl + 30 mM $CaCl_2$) though the process was slower at higher salinity. The presence of Ca^{+2} had no positive effect on the process of germination. The present observations confirm that the presence of salt in the growth media has little adverse effect on the process of germination of the seeds of leguminous plants (Imamul Huq and Larher, 1983 a, 1983 c), at least when the salts are present in vermiculite used as growth media.

Increases in fresh and dry weights of the plants in the presence of up to 50 mM $NaCl_{ext}$ suggests that this species might benefit from Na^+. This might probably be due to a stimulatory effect of Na^+ on some enzymes (Turner and Turner, 1980) or to an osmoregulatory role partially replacing K^+. However, the decrease in plant height under saline conditions is a common phenomenon with leguminous plants (Imamul Huq and Larher, 1983 a, 1983 c; Nukaya et al., 1982 a, 1982 b) and is principally associated with a slower rate of cell division (Nieman, 1965).

Growth reduction in non halophytes in the presence of high $NaCl_{ext}$ might result from a passive accumulation of Na^+ which creates an «ion excess» effect (Greenway and Munns, 1980) due to an imbalance in the external Na/Ca ratio. The idea that this phenomenon is supposed to be avoided by maintaining the external Na/Ca ratio at a constant level with changing $NaCl_{ext}$ concentrations (Epstein, 1980; Greenway and Munns, 1980) was confirmed by our observations. The adverse effects of $NaCl_{ext}$ on all the growth parameters studied were significantly alleviated by maintaining a constant Na/Ca ratio. This might have been due to a better Ca^{+2} status of the plants, leading to better cell division and extension (Clarkson and Hanson, 1980).

Salt stress in non halophytes might also induce some kind of water deficit in the expanded tissues (Greenway and Munns, 1980) causing growth reduction. In the present study it was observed that the relative water content (RWC) of the leaves of the salt-treated plants (figs. 4 a and 4 b) were lower than those of the corresponding control plants. The measured growth parameters however, were better in the salt treated plants. This was more pronounced when a constant Na/Ca ratio was maintained which clearly indicates that the growth reduction observed in the non halophyte in the presence of excess NaCl is mainly attributable to the «ion excess» effect.

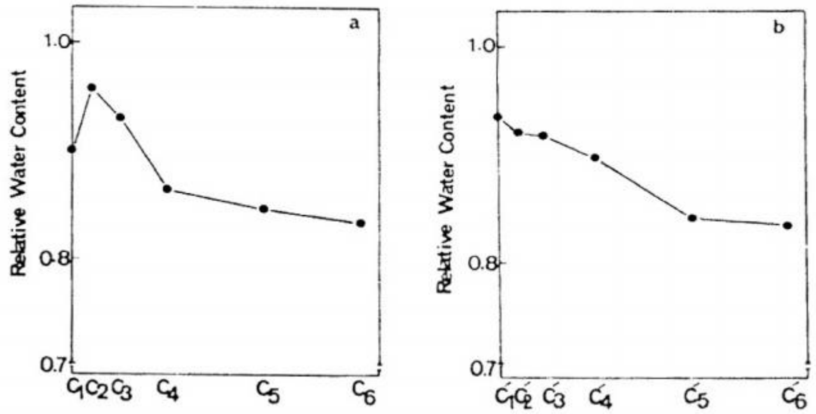

Fig. 4: Relative water contents of the plants at harvest as modified by different concentrations of $NaCl_{ext}$ (a) or by $NaCl_{ext}$ when Na/Ca_{ext} was kept constant at 5.0 (b). $C_1 \ldots C_6$ and $C'_1 \ldots C'_6$ represent the treatments as described in figure 1.

Concerning the ion balance, the plants of *V. sinensis* behaved like other non halophytes in accumulating Na^+ at the expense of K^+, thereby creating a condition of K-deficiency at higher salinity. This can be associated with an increased accumulation of, among other solutes, amides and amino acids. K^+ deficiency in various plants has been found to induce an accumulation of asparagine as well as the diamine putrescine (Stewart and Larher, 1980; Smith et al., 1982). However, a concurrent uptake of an appreciable quantity of K^+ in the presence of a $NaCl_{ext}$ concentration as high as 50 mM might suggest some kind of adaptation of this species towards NaCl salinity. The maintenance of appreciably high amounts of Ca^{+2} in the media with a concomitant increase in salinity completely changed the situation of endogenous Na-K balance. Under such conditions, Na^+ in no case, exceeded the K^+ contents, especially in the above-ground parts. This is presumably related to the well known influence of Ca^{+2} on the maintenance of the membrane integrity thereby excluding a very high non-metabolic uptake of Na^+. However, at high NaCl, an invasion of tissues by Na^+ can not be totally prevented. In addition, as the Ca^{+2} status was improved in the plants, this too, might have exerted an influence on the stability of a variety of cytoplasmic structures and enzymes (Clarkson and Hanson, 1980), ultimately improving the salt resistance of the plants.

The anion status, especially of NO_3^- and $PO_4^{-3}{}_4$, was greatly modified by the presence of Ca^{+2}. The NO_3^- concentrations are always found to decrease with increasing accumulation of Cl^- under salinity (Imamul Huq and Larher, 1983 a), but the situation was modified to a greater extent in the presence of Ca^{+2}, where the plants accumulated more NO_3^- than the control plants in the presence of $NaCl_{ext}$ as high as 50 mM. The plants were found to maintain a substantial quantity of NO_3^- in their tissues even under strong salinity suggesting that NO_3^- might play a significant role as an osmoregulator.

Accumulation and transport of the low molecular weight organic compounds considered to be either compatible solutes or stress markers (Hsiao, 1973; Stewart and Larher, 1980; Imamul Huq and Larher, 1983 b) were also modified by the presence of higher Ca^{+2}. Both amides and amino acids, solutes characteristics of a stress condition (Stewart and Larher, 1980; Imamul Huq and Larher, 1983 b), decreased in the roots. This can be related to a better transport of the reduced nitrogen to the shoots thus giving better growth, which was certainly due to the ameliorative effect of Ca^{+2} on the harmful influence of excess Na^+. Proline accumulation in the plants deserve special mention. If this amino acid is considered to be a stress marker, it is evident from the present study that the plants of *V. sinensis* can tolerate moderate degrees of NaCl salinity and even more by maintaining a proportionately high Ca^{+2} with increasing salinity. Consistent with our previous results with another species of leguminous plant (Imamul Huq and Larher, 1983 b), the plants of *V. sinensis* also accumulated QACs in response to salinity. It remains, however, to justify the presence of these compounds as compatible solutes in salt stressed leguminous plants since the non stressed plants also contain substantial amounts. Such a phenomenon is also observed in halophytes (Wyn Jones, 1980). However, the magnitude of the level of QACs in non-treated plants and that of the response to salinity are different. In leguminous plants, the accumulation of QACs is presumably of secondary importance in the adaptation to NaCl salinity.

The fact that the major osmotica of glycophytes are, in addition to K salt, sugar (Hellebust, 1976) was also evident in the present study. The better their sugars status, the better the plants of cowpea adjusted osmotically to salinity. Increase in the quantity of total polyols under salinity could also be of significance as regards the osmoregulation process in *V. sinensis* and may be linked to some general phenomena: significant amounts of different kinds of polyols have been reported to occur in leguminous plants (Ford, 1982; Smith and Phillips, 1982).

It is certain that as the plants survived the presence of high concentration of salt (150 mM NaCl + 30 mM $CaCl_2$), they must have adjusted osmotically to this adverse situation. When the contributions of the principal osmotica were evaluated in relation to endogenous Na^+, it became clear that K^+ and NO_3^- played the key role at lower Na^+ values, sugars and polyols at higher concentrations. However, the involvement of Na^+ in this situation is probably necessary since the concentrations of these organic solutes were too low to lower the internal potential. The increase in proline and QACs with decreasing K^+, NO_3^-, or even sugars, indicates that they might also play a part in this process, but the magnitude of the response at their levels is also too low to be of real adaptative value.

In conclusion, it can be said that the plants of *V. sinensis* tolerated $NaCl_{ext}$ concentrations as high as 150 mM with an ultimate growth reduction which could be effectively ameliorated by maintaining a proportionately high Ca_{ext}^{+2} within the range of 10 to 20 mM (in the presence of the said concentration of NaCl). However, Ca^{+2} concentrations higher than 20 mM in the presence of high $NaCl_{ext}$ (e.g., 150 mM) might introduce some kind of toxic «ion excess» effect.

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