J. Plant Physiol. Vol. 136. pp. 439–443 (1990)

Effects of the Sodium/Calcium Ratio in Modifying Salinity Response of Pigeonpea (*Cajanus cajan*)

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Submitted as JA No. 933 by ICRISAT.

Received August 5, 1989 · Accepted February 25, 1990

Summary

There was a positive growth response by salt-tolerant (ICPL 227) and salt-sensitive (HY 3 C) pigeonpea genotypes to a decreasing Na/Ca ratio under constant salinity. The relative growth differences between tolerant and sensitive genotypes persisted at different Na/Ca ratios at 6 and 8 dS m^{-1} salinity levels. A decrease in the Na/Ca ratio under salinity enhanced K uptake and reduced Na uptake, thus increasing the K/Na ratio. The K/Na ratio in shoots of the tolerant genotype was greater than in the sensitive genotype at different Na/Ca ratios at both salinity levels. The tissue Cl levels increased with decreasing Na/Ca at both salinity levels. This increase was greater in the sensitive than in the tolerant genotype. Thus relative growth differences and differences in Na, K and Cl uptake between tolerant and sensitive genotypes persisted across the range of Na/Ca ratios. This suggests that results of screening for genotypic differences at one particular Na/Ca ratio would be applicable to other Na/Ca ratios.

Key words: *Cajanus cajan, calcium, chloride, ionic relations, plant growth, salinity tolerance, sodium.*

Abbreviations: DAS = days after sowing; ECe = electrical conductivity; dS m^{-1} = decisiemens per meter.

Introduction

Pigeonpea (*Cajanus cajan* [L.] Millsp.) is an important grain legume of semi-arid regions where salinity problems can be acute. Over 90% of the world's pigeonpea is produced in India, where salinity problems are becoming increasingly severe (Chauhan, 1987). An estimated area equivalent to 33% of irrigated land world-wide is affected by salinity (Carter, 1975). It is often argued that genetic improvement of salinity tolerance for crop plants should supplement reclamation and drainage as part of an integrated approach towards economic utilization of salt-affected soils.

In saline soils, Na and Ca are usually present in 2:1 to 6:1 molar ratios (Shannon, 1984), except in a few cases where soil salinity is associated with alkalinity. In view of the widely accepted role of Ca in membrane stabilization and selectivity in ion uptake, and also considering the reports that presence of Ca in the medium can enhance salinity tolerance of several crop species (Hyder and Greenway, 1965; Lahaye and Epstein, 1971; Marcar, 1986), this investigation was conducted to ascertain the role of Ca in salinity tolerance for pigeonpea and its influence on ionic relations. From our earlier studies on the evaluation of pigeonpea genotypes (Subbarao, 1984), significant differences in their tolerance to salinity were observed. Since salinity under field conditions is a complex problem and the relative Na and Ca concentrations vary spatially, genotypes selected for saline conditions should ideally be able to perform uniformly across a range of Na/Ca concentrations. A tolerant (ICPL 227) and a sensitive (HY 3 C) pigeonpea genotype were selected for this

Table 1 Required amounts of NaCl and $CaCl_2$ in various Na/Ca

Salinity level		Calcium concentration (mM)	NaCl + $CaCl_2$ (mM)	Na/Ca
I Control		0 36		
II 6 dS m^{-1}	a	0 36	59 3 + 0 36	165
	b	1 00	58 0 + 1 00	58
	c	5 00	50 0 + 5 00	10
	d	10 00	40 0 + 10 00	4
	e	15 00	30 0 + 15 00	2
III 8 dS m^{-1}	a	0 36	79 3 + 0 36	220
	b	1 00	78 0 + 1 00	78
	c	5 00	70 0 + 5 00	14
	d	10 00	60 0 + 10 00	6
	e	15 00	50 0 + 15 00	3
	f	20 00	40 0 + 20 00	2

study to determine their relative growth responses and ion uptake behavior across a range of Na/Ca concentrations at salinity levels found critical for the growth of pigeonpea

Materials and Methods

The pigeonpea genotypes selected for this study, ICPL 227 and HY 3C, were obtained from the Pigeonpea Breeding Unit, Legumes Program, ICRISAT, India Pigeonpea seeds were surface sterilized with 0 2 % $HgCl_2$ solution for 5 min and then thoroughly washed with deionized water and soaked overnight The growth medium consisted of sieved river sand, washed and soaked in acid solution (pH 1 to 2) for 24 h, and then thoroughly washed with tap water, dried, and filled in 180 mm diameter polypropylene pots (2 5 kg sand pot^{-1}) Eight pigeonpea seeds of either genotype were sown per pot The sand surface in each pot was covered with 50 g of polythene beads to minimize evaporation Seedlings were thinned to four per pot at 10 DAS Plants were given deionized water up to 13 DAS Two salinity levels, 6 and 8 dS m^{-1}, with different Na/Ca ratios (Table 1) were imposed at 14 DAS by flushing each pot with 1 L of treatment solution For preparing treatment solutions, 25 % strength modified Arnon and Hoagland solution supplemented with 1 79 mM NH_4NO_3 and various levels of NaCl and $CaCl_2$ were used to obtain the required Na/Ca ratio for treatment and electrical conductivity (Table 1) Calcium was substituted for Na up to a maximum of 50 % Na so as to maintain the electrical conductivity (ECe), osmotic potential, and Cl concentrations constant within a salinity level irrespective of the Na/Ca status of the treatment The composition of the nutrient solution was (mM)· KH_2PO_4 (0 23) KCl (0 52), $MgSO_4$ (0 25), $CaCl_2$ (0 37), $MnSO_4$ (0 0015), $ZnSO_4$ (0 00023), $CuSO_4$ (0 00025), H_3BO_3 (0 001), Na_2MoO_4 (0 00005) and NaFeEDTA (0 04) For the control treatment, the nutrient solution alone (ECe 0 33 dS m^{-1}) was used for flushing.

The experiment was conducted as a randomized complete block design with four replications in a greenhouse where the temperature was maintained at 28/22 °C (day/night) and the relative humidity at 60–70 % At the end of each day, the evapo-transpirational losses were adjusted by adding deionized water after weighing the pots Every four days, pots were flushed with treatment solutions (250 ml pot^{-1}) to avoid salt accumulation and rerandomized to minimize spatial effects in the greenhouse Plants were grown to 50 DAS

At harvest, leaf area was measured with an automatic leaf area meter (Delta T Devices Limited, England) Fallen leaves were collected and included for dry matter determination and chemical analysis For determination of Na, K and Ca, finely ground samples of 200–300 mg were digested with 6 mL of concentrated nitric acid, sulphuric acid, and perchloric acid (10 0 5 2) on a sand bath at 250 °C for 6 to 8 h (Piper, 1952) The digested plant samples were diluted and analyzed for various elements by atomic absorption spectrophotometry (Varian, Model 1200) Chloride content in the plant samples was determined by Mohr's volumetric method (Blaedel and Meloche, 1960)

Results and Discussion

Shoot dry matter significantly increased with decreasing Na/Ca ratio in the medium at 6 and 8 dS m^{-1} salinity levels (Fig 1) A similar trend was observed for leaf area and root dry matter, although the data are not presented here The positive growth response to Ca under saline conditions corroborates previous studies on barley (Hyder and Greenway, 1965), *Phaseolus vulgaris* (Lahaye and Epstein, 1971), Wimmera rye grass (Marcar, 1986) and sorghum (Grieve and Maas, 1988) Although growth was improved in both genotypes, it remained significantly higher in the tolerant geno-

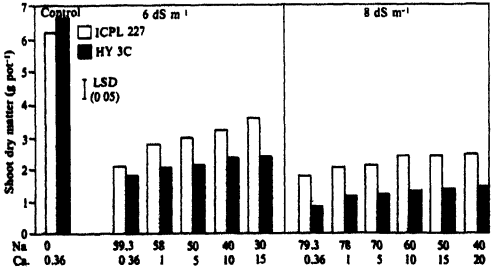

Fig. 1 Effect of Na/Ca ratio at 6 and 8 dS m^{-1} salinity levels on shoot dry matter of pigeonpea genotypes ICPL 227 and HY 3C. Data are means of four replications

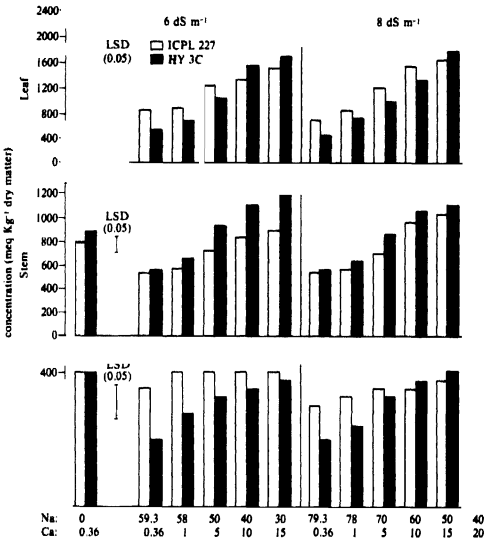

Fig. 2: Effect of Na/Ca ratio at 6 and 8 dS m^{-1} salinity levels on leaf, stem and root Ca concentration of pigeonpea genotypes ICPL 227 and HY 3C. Data are means of two replications.

type ICPL 227 at all Na/Ca ratios at both salinity levels than in the sensitive genotype HY 3C.

The tissue (shoot and root) Ca concentration was less than that of the control in saline media when the external Ca concentration was maintained at 0.36 mM (Fig. 2). It increased with decreasing Na/Ca ratio in the medium at both salinity levels. This indicates that Ca uptake is affected under saline conditions when the Na/Ca ratio is high (Gerard and Hinojosa, 1973; Lynch and Läuchli, 1985; Cramer et al., 1987). This decrease in leaf and root Ca level at high Na/Ca ratios was significantly greater in the sensitive genotype than in the tolerant one. Similar observations were made by Elzam and Epstein (1969) in *Agropyron elongatum* and *A. intermedium* where the differences in their tolerance for salinity (NaCl) were associated with differences in their calcium uptake.

Shoot (leaf and stem) sodium concentrations decreased with decreasing Na/Ca ratio at 6 and 8 dS m^{-1} salinity levels Fig. 3). This could be a result of both decreasing Na concentrations in the medium and increasing Ca, the latter maintaining membrane integrity (Lahaye and Epstein, 1971; Cramer et al., 1987). The tolerant genotype ICPL 227 maintained lower tissue Na levels compared to the sensitive genotype HY 3C at different Na/Ca ratios at both salinity levels. The relative differences increased at lower Na/Ca ratios (part from the lowest Na/Ca ratio at 6 dS m^{-1}).

Shoot (leaf and stem) K concentrations increased with decreasing Na/Ca ratio in the medium at both salinity levels and in both genotypes (Fig. 4), as reported for other species (Jacobson et al., 1961; Rains and Epstein, 1967; Elzam and Epstein, 1969; Cramer et al., 1985; Kent and Läuchli, 1985). The K concentrations in leaf tissue were significantly higher in the tolerant genotype than in the sensitive genotype in all treatments, but such differences in stem tissue were only apparent at lower Na/Ca ratios. Due to enhanced K uptake and reduced Na uptake the tissue K/Na ratio increased with decreasing Na/Ca ratio. This phenomenon can be attributed either to reducing Na concentration in the medium or to an effect of Ca in enhancing K/Na selectivity.

Tissue (leaf and stem) Cl concentration increased with decreasing Na/Ca ratio in the medium at both salinity levels (Fig. 5), even though Cl concentration in the medium within a salinity level was constant across Na/Ca ratios. This increase in shoot Cl concentration with decreasing Na/Ca ratio was significantly higher in the sensitive genotype than in the tolerant genotype. Although calcium enhances the uptake of several anions, nitrate, bromide, chloride, and sulphate, under non-saline conditions (Hooymans, 1964), reports of Cl enhancement under saline conditions have not come to our attention. The enhancement is probably a consequence of maintenance of cation-anion balance: total cat-

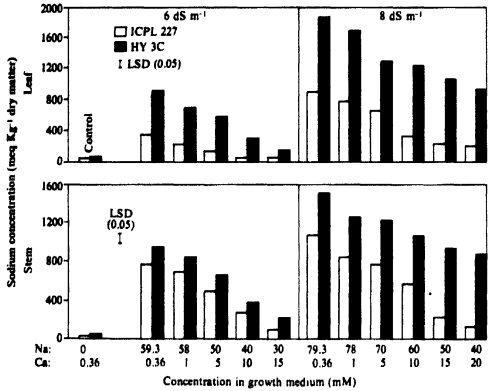

Fig. 3: Effect of Na/Ca ratio at 6 and 8 dS m^{-1} salinity levels on leaf and stem Na concentration of pigeonpea genotypes ICPL 227 and HY 3C. Data are means of two replications.

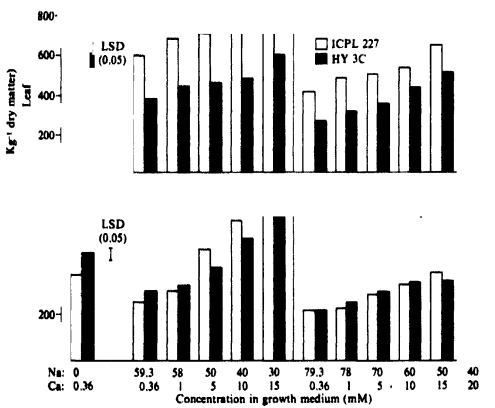

Fig. 4: Effect of Na/Ca ratio at 6 and 8 dS m^{-1} salinity levels on leaf and stem K concentration of pigeonpea genotypes ICPL 227 and HY 3C. Data are means of two replications.

ion (Ca + Na + K) concentrations increase with decreasing Na/Ca ratio (Figs. 2–4) in a similar manner to the increase in Cl concentration (Fig. 5). It is suspected that the enhancement of Cl uptake negates the positive effects of decreasing Na/Ca ratios in the medium in increasing K/Na ratio in the shoot under saline conditions.

In conclusion, a positive growth response of pigeonpea genotypes to decreasing Na/Ca ratio at constant salinity levels is indicated. This response is attributable to high K/Na ratios in plant shoots, resulting from both decreasing Na concentrations in the medium and from the effect of Ca in enhancing K/Na selectivity. However, growth rates may to some extent be counteracted by the enhancement of Cl uptake at lower Na/Ca ratios. The salt-tolerant genotype ICPL 227 was better able to exclude Na and Cl from, and maintain higher K/Na ratios in, the shoot compared to the salt-sensitive genotype HY 3C. Relative growth differences and, to a large extent, differences in Na, K, and Cl uptake between tolerant and sensitive genotypes persisted across the range of Na/Ca ratios. This study is therefore of practical

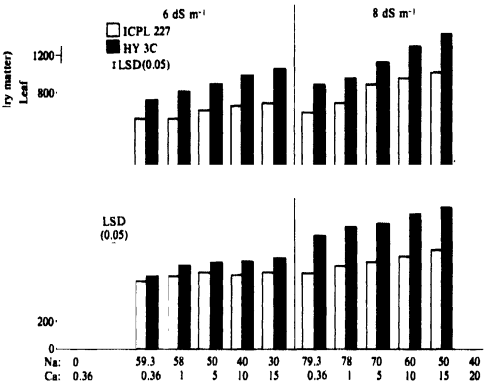

Fig. 5: Effect of Na/Ca ratio at 6 and 8 dS m^{-1} salinity levels on leaf and stem Cl concentration of pigeonpea genotypes ICPL 227 and HY 3C. Data are means of two replications.

importance, since relative Na/Ca concentrations may vary spatially across saline fields, and genotypes selected for saline conditions should perform uniformly across a range of Na/Ca levels.

Acknowledgements

We are indebted to D. L. Oswalt and F. B. Lopez, ICRISAT, for their constructive criticism of this manuscript.

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J Plant Physiol. Vol 136. pp. 444–450 (1990)

Prolyl 4-Hydroxylase from *in vitro* Cell Cultures

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Received December 12, 1989 Accepted February 25, 1990

Summary

Fifteen cell suspension strains cultivated *in vitro* were screened for the highest cell wall hydroxyproline content. Differences in hydroxyproline content were observed in different plant families as well as in different strains of a same family. The suspension of soybean *Glycine max* cv Mandarin showing the highest hydroxyproline content was chosen as a source of prolyl 4-hydroxylase (EC 1 14 11.2.) for purification. Kinetics of hydroxyproline content and prolyl 4-hydroxylase specific activity were similar, with a maximum at 14 days after subculture. Elicitation of cell suspensions by arachidonic acid leads to an increase in 30 % of activity

Triton X-100 was the most effective detergent among four non-ionic detergents tested for solubilizing the soybean membrane-bound prolyl 4-hydroxylase from microsomal fractions This enzyme, unstable at room temperature and at 4 °C even with the addition of enzyme co-factors (ascorbic acid and ferrous ions), can be stored for several months at −80 °C.

Prolyl 4-hydroxylase was purified from microsomal fractions through chromatography on DEAE-Sephadex A-25 and poly L-proline-Sepharose affinity columns

Comparison of prolyl 4-hydroxylase activities from different forms of soybean (cell suspensions, cell suspensions elicited with arachidonic acid, calluses, whole plant) shows that activity is highest in the cell suspensions.

Key words: Carthamus tinctorius, Catharanthus roseus, Choisya ternata, Daucus carota, Eschscholtzia californica, Helianthus annuus, Ochrosia elliptica, Papaver somniferum, Phaseolus vulgaris, Glycine max, hydroxyproline, prolyl 4-hydroxylase.

Introduction

Hydroxyproline is widely distributed in plants, where it is found in high levels in arabinogalactan proteins (Fincher et al., 1983) and in the hydroxyproline-rich cell wall glycoproteins (Lamport, 1973).

Hydroxyproline-rich cell wall glycoproteins may have a structural and developmental function in plants (Varner, 1983) similar to that of collagen in animals, or play a role during recognition processes (Lis et al., 1981; Leach et al., 1982). An increase in plant cell wall hydroxyproline has been noted when cell elongation ceases (Cleland et al., 1967) or is induced to cease (Ridge et al., 1970). These changes are also observed in diseased plants, where they are part of the defense mechanisms against pathogen attack (Toppan et al., 1982), or in elicitor-treated bean cell cultures (Dixon et al., 1986).

Hydroxylation of proline is a post-translational modification catalyzed by prolyl 4-hydroxylase (proline: 2 oxoglutarate dioxygenase EC 1.14.11.2.).

Plant prolyl 4-hydroxylases have been studied less compared with the animal enzyme (Chrispeels, 1984), and a better knowledge of the involvement of prolyl 4-hydroxylase in the defense mechanism is necessary. As a first step to investigate this protein, we screened for the best source of this enzyme to have a sufficient quantity for characterization.

Because the enzyme is normally present in low levels in plants, suspension-cultured cell strains were screened for their cell wall hydroxyproline content, which we assumed to be positively correlated with prolyl 4-hydroxylase activity.