Genotypic Variation in Salinity Response of Chickpea and Pigeonpea

C. JOHANSEN, N.P. SAXENA, Y.S. CHAUHAN, G.V. SUBBA RAO, R.P.S. PUNDIR, J.V.D.K. KUMAR RAO AND *M. K. JANA

International Crops Research Institute for the Semi-Arid Tropics, Patancheru P.O., Andhra Pradesh 502 324, India
*Indian Institute of Technology, Kharagpur, West Bengal 721 302, India

Summary

Variation among 81 genotypes of chickpea in salinity response measured in pot culture was small. Electrical conductivity (1:2 soil-water extract) at 50% reduction of shoot weight was within a range of 1.2 dS/m for any experimental run. Among a set of wild species related to chickpea, some lines showed even greater sensitivity to salinity than cultivated chickpea but none showed greater tolerance. Thus, in the chickpea material so far tested, there are no substantial sources of salinity tolerance that would warrant incorporation in a breeding programme. For a range of cultivated pigeonpea genotypes, there was limited variability in salinity response when compared in pot culture but variability was considerable when compared in a solution culture system. Further, some wild relatives of pigeonpea had markedly better tolerance than the best cultivated pigeonpea control. Thus there is some scope for improving the salinity tolerance of pigeonpea.

Introduction

Soil salinity can be a severe constraint for growth and yield of chickpea *(Cicer arietinum* L.) and pigeonpea *[Cajanus cajan* (L.) Millsp.] in environments otherwise conducive to these crops. A major example is in the Indian subcontinent (Chauhan, 1987). Although management remains the most feasible means of improving crop yields on salt-affected soils, particularly in irrigated areas with inadequate provision for drainage, there is scope for genetic enhancement of salt tolerance in particular crops (Epstein, 1985; Epstein & Rains, 1987). Attempts were made to assess the extent of genotypic diversity in response to salinity for chickpea and pigeonpea, as a prerequisite to identifying sources of salinity tolerance for use in breeding programmes.

Many of the initial studies to identify genotypic differences in salinity tolerance were conducted in saline fields (Chauhan, 1987). However, as found earlier (Richards, 1983) the heterogeneous nature of saline soils prevented unequivocal quantification of genotypic differences. Thus screening was conducted under more uniform and controlled conditions, such as in pot or solution culture in the greenhouse or controlled environment chamber. However the limitations of these techniques, such as inability to account for effects on grain yield and salt distribution in a soil profile, are recognised, but they were utilized as a first approximation to quantifying genotypic differences in response of plant growth to salinity.

Materials and Methods

Chickpea

Four pot tests were conducted in the

greenhouse. Day/night temperatures were 30°-35°/22°-26° C, with day temperatures maintained by evaporative coolers. Relative humidity during the day was 60-70% and the light intensity was about 80% of natural sunlight.

The tests were conducted in plastic pots (13 cm top diameter) containing 1.5 kg of 2 mm sieved Vertisol. The cation exchange capacity (CEC) of these Vertisols was 40 milliequivalents/100 g of soil and the Ca^{2+} saturation in the profile of 0.90 cm ranged between 62 and 71%. The electrical conductivity (EC) of the soil used in these pot experiments with Vertisol (including the experiment with pigeonpea, mentioned later) was measured as a 1:2 soil-water extract (SWE). For these Vertisols, the relationship of EC in soil saturation extract (SSE) and SWE is:

$$\text{EC in SSE} = -2.191 + (4.012 \times \text{EC in 1:2 SWE}),\ r^2 = 0.985 \quad (1)$$

This relationship allows comparison of EC measured in Vertisol with that measured in sand or solution culture experiments.

Five salinity levels were created by mixing anhydrous salts of NaCl, Na_2SO_4 and $CaCl_2$ in the ratio 7:1:2. This mixture gives a salt composition similar to that of natural saline Vertisols. The salinity treatments were — control (the EC of the soil lot used), 1,2,3 and 5 dS/m (but 4.5 instead of 5 dS/m in Test 2). The treatments were arranged in a split plot design, with salinity levels in main plots and genotypes in subplots. There were 2 replications. Some details of the tests conducted are given in Table 1. Tests 1-3 comprised cultivated chickpea genotypes and test 4 wild species of *Cicer.*

No basal nutrients were added as other pot tests indicated no further nutrient requirements for seedling growth of chickpea in this soil. Pots were not inoculated with rhizobia as native rhizobial populations in this soil ensured good nodulation in control treatments. Six seeds were sown in each pot and seedlings thinned to 3 uniform plants per pot at the 3-4 leaf stage. Pots were maintained near field capacity during growth. Salinity level in the pots was again determined 20 days after sowing and any reduction in salinity level was restored by irrigating with saline solution of the required EC. At harvest, shoot dry weight was recorded.

Pigeonpea

Pot Experiment — This was conducted in a greenhouse with day/night temperatures 28°-30°/22°-24° C, relative humidity 60-70% and 80% natural light intensity. Plastic pots (13 cm top diameter) filled with 1 kg of sieved Vertisol were used. A basal dose of 1.125 g single superphosphate per kg soil was applied and seeds were inoculated with *Rhizobium* strain IC 3195.

Twenty seven pigeonpea genotypes were sown on 14 February 1986 at 10 seeds/pot and seedlings thinned to 4 plants/pot at 8-9 days after sowing. After thinning, 5 salinity levels were imposed: control (0.46), 1.5, 3, 4.5 and 6.0 dS/m (EC in 1:2 SWE). Saline solutions containing NaCl, $CaCl_2$ and $MgSO_4.7H_2O$ in the proportion of 2:1:1, again of similar composition to local saline Vertisols, were added in 3 split applications. Pots were maintained at 25% water content, which is below the field capacity of this soil (about 30%).

The trial was laid out in split plot design

Table 1. Details of genotypes and sowing and harvest dates of the pot tests screening chickpea genotypes for salinity response

Test	Number of test entries	Control cultivars	Sowing date	Harvest date
1	29	Annigeri, K 850, G 130	8-8-85	13-9-85
2	29	Annigeri, K 850, G 130, L 550	27-9-85	6-12-85
3	29	Annigeri, K 850, L 550	22-1-86	1-4-86
4	13	Annigeri, L 550, G 130	22-1-86	2-4-86

with salinity treatments in main plots and genotypes in sub-plots. There were 3 replications. Plots were rerandomized at 10-day intervals. Shoot dry weight was determined after harvest at 40 days from sowing.

Solution Culture — This experiment was conducted in a controlled environment chamber under the following conditions: day/night temperature 30°±2°/22°±2° C; photoperiod 14 hr; irradiance (400-700 nm PAR) 400 $\mu E/cm^2/s$; relative humidity 70-80%. Plants were grown in 100 L perspex tanks in which salinity levels of either 0, 5, 6, 7, 8 or 9 dS/m were imposed. A strip plot experimental design was used with salinity treatment as the non-replicated main plot and genotypes, replicated 4 times, in subplots.

Sixteen pigeonpea genotypes used in this study were chosen on the basis of divergent responses from an earlier mass screening for salinity tolerance in solutions of 6 dS/m EC.

Seeds were surface sterilized with a 0.2% $HgCl_2$ solution for 5 min, washed several times with deionized water and soaked in water overnight. Soaked seeds were placed in growth pouches perforated to permit aeration and the pouches placed in the tanks containing 60 L deionized water. The blotting paper of the pouches was brought into contact with the nutrient solution in the tank by slitting the bottoms of the polythene bags of the pouches. Germinating seeds were covered with a black cotton cloth and exposed to light on the sixth day, after complete seedling emergence. The solutions in the tanks were uniformly aerated throughout the experiment. On the seventh day, seedlings were thinned to 5 per pouch. On the tenth day, the deionized water in the tanks was replaced by 100 L of the treatment/nutrient solutions.

A half strength modified Arnon and Hoagland solution of the following composition was used (ppm): N as NH_4NO_3, 20; KH_2PO_4, 6: KCl, 77.5; $MgSO_4.7H_2O$, 125; $CaCl_2.2H_2O$, 107.5; $MnSO_4.H_2O$, 0.5; $ZnSO_4.7H_2O$, 0.125; $CuSO_4.5H_2O$, 0.125; H_3BO_3, 0.125; $Na_2MoO_4.2H_2O$, 0.025; $FeC_6H_5O_7.5H_2O$ (Ferric citrate), 15. pH was maintained at 6-7 during the experiment. Salinity treatments were imposed by adding NaCl + $CaCl_2.2H_2O$ (w/w, 1:1) to obtain the required EC. Solutions were monitored daily for EC and adjustments made accordingly. Treatment/nutrient solutions were renewed every 10 days. The nitrogen level was increased to 50 ppm on the 30th day. Plants were harvested for dry matter estimation at 40 days after sowing.

Sand Culture — This experiment was conducted in a glasshouse under the same environmental conditions as for the pigeonpea pot experiment, except that the minimum temperature range was 18°-20° C. Plants were grown in plastic pots with 15 cm top diameter and filled with sieved river sand that had been washed 5-6 times with tap water, soaked in an acid solution (pH 1-2) for 24 hr, again washed 5-6 times with tap water, and dried. The pots containing washed sand were steam sterilized for 1 hr.

Genotypes tested were wild species related to cultivated pigeonpea, *Atylosia platycarpa, A. scarabaeoides, Rhynchosia albiflora, Dunbaria ferruginea,* together with salinity tolerant (ICPL 227) and susceptible (HY 3C) pigeonpea cultivars as controls. Seeds of the wild species required special treatment to ensure quick and even germination; viz. scarification by nicking of the testa and incubation in moist blotting paper at 28° C for 3-5 days. Pigeonpea seeds were surface sterilized with 0.2% $HgCl_2$, washed with distilled water and soaked in deionized water overnight.

Germinating seeds were sown at 8 seeds per pot and seedlings thinned to 4 per pot after 10 days. For the first 14 days the pots were watered with deionized water only. The sand surface was covered with polythene beads to minimize evaporation. Four days after thinning the following treatments were imposed, using the same salt mix as for the solution culture experiment: 0, 4, 6, 8, 10 and 12 dS/m. A quarter-strength modified Arnon and Hoagland nutrient solution (i.e. half the concentrations given for the solution culture experiment) with 50 ppm NH_4NO_3 was used. The experiment was laid out in randomized

block design with 4 replications and pots were rerandomized every 3 days.

When imposing salt treatments to levels above 4 dS/m, levels were successively increased by 2 dS/m each day to avoid "salt shock". Pots were flushed with respective treatment solutions (250 ml/pot) on alternate days and evaporational water losses compensated by deionized water. Plants were harvested for dry matter determination at 45 days after sowing.

Results

Salinity response between genotypes was compared by arbitrarily defining a critical EC as that EC causing a 50% reduction in shoot growth, as compared with a control treatment to which no salt is added. In 3 experiments comparing chickpea genotypes there was little variation in salinity response among the genotypes (Fig. 1a - c). Critical EC (1:2 SWE) differed by slightly >

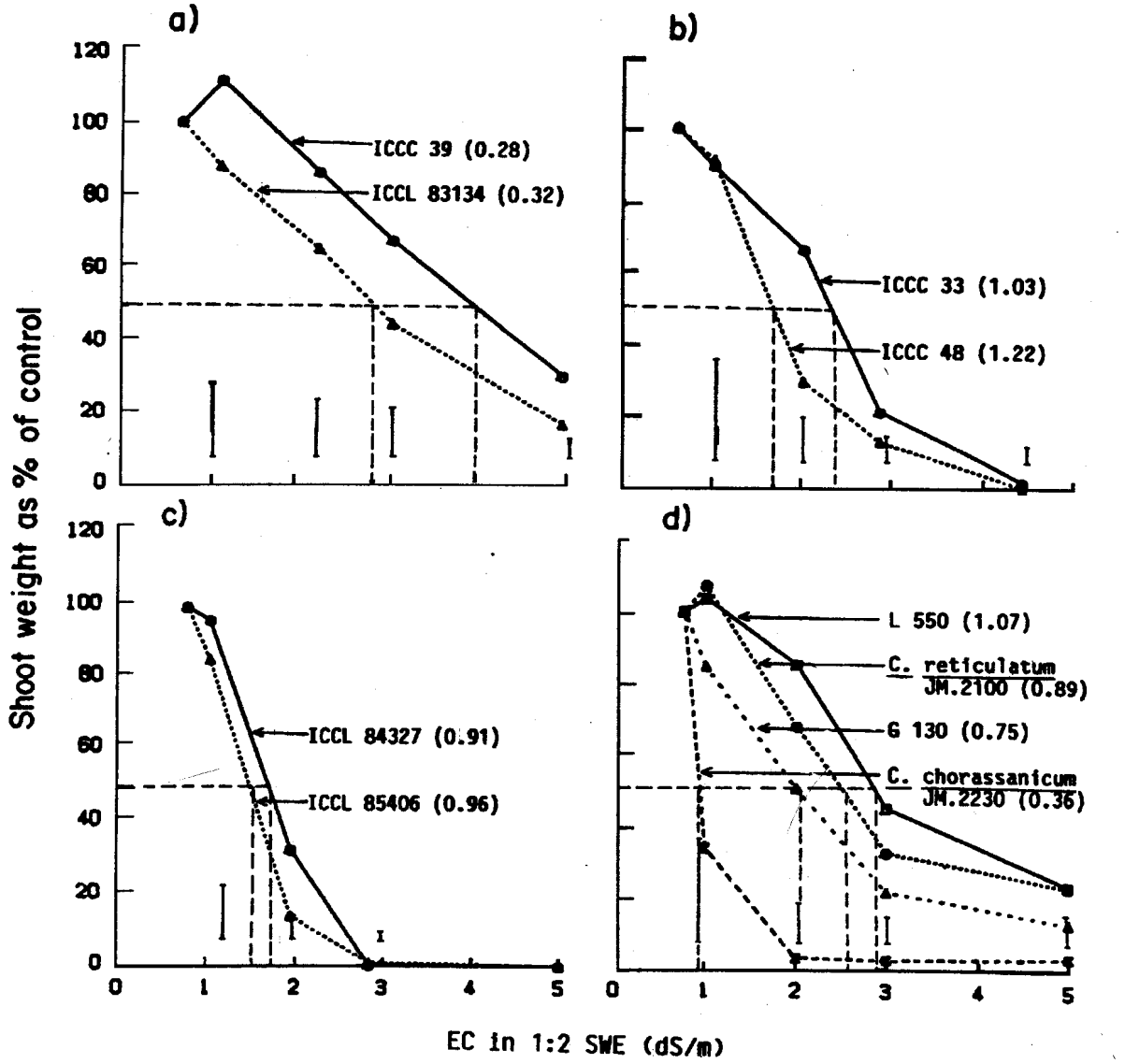

Fig. 1. The effect of salinity (EC) on the shoot weight of chickpea genotypes and related wild species. Figs. 1a-d refer to Tests 1-4, respectively. The most- and least-responsive genotype in a particular experiment is presented in each figure. In (d), the most- and least-sensitive wild species is presented together with two cultivated chickpea controls. Shoot weights (g/plant) for the control treatment are indicated in parentheses for each genotype presented. Standard errors of mean of all genotypes tested are shown for each salinity level.

1 dS/m in Test 1 (Fig. 1a) but in Test 3 (Fig. 1c) the variation was well within 0.5 dS/m.

There was greater variation in salinity response among the wild species tested but the variation was towards greater salt sensitivity (Fig. 1d). *Cicer reticulatum* (JM 2100) was the most tolerant of the wild species tested but its salinity response was similar to that of the chickpea cultivars.

It was noted that critical EC differed considerably between experiments, for the entire group of genotypes under test or for control entries common between experiments. For example, critical EC was in the vicinity of 3-4 dS/m in Test 1 but only around 1.5 dS/m in Test 2.

For the pigeonpea genotypes tested in potted Vertisol, the variation in critical EC (1:2 SWE) was between 1.5 and 3 dS/m (Fig. 2a). Critical EC for cultivated pigeonpea in solution and sand culture, was around 5-7 dS/m (Fig. 2b, c), in accordance with equation 1. In solution culture, a greater variability between genotypes in salinity response was apparent (Fig. 2b). Genotypes HY 3C and HY 4 were most susceptible and ICP 8626 and ICPL 227 were most tolerant; the remaining genotypes under test showed an intermediate response (e.g. ICP 8698). In solution culture, all genotypes died at 8 and 9 dS/m.

The differences in response between the tolerant ICPL 227 and the susceptible HY 3C were also apparent in sand culture (Fig. 2c). These differences have been repeatedly demonstrated in various solution, sand, and soil culture experiments as these geno-

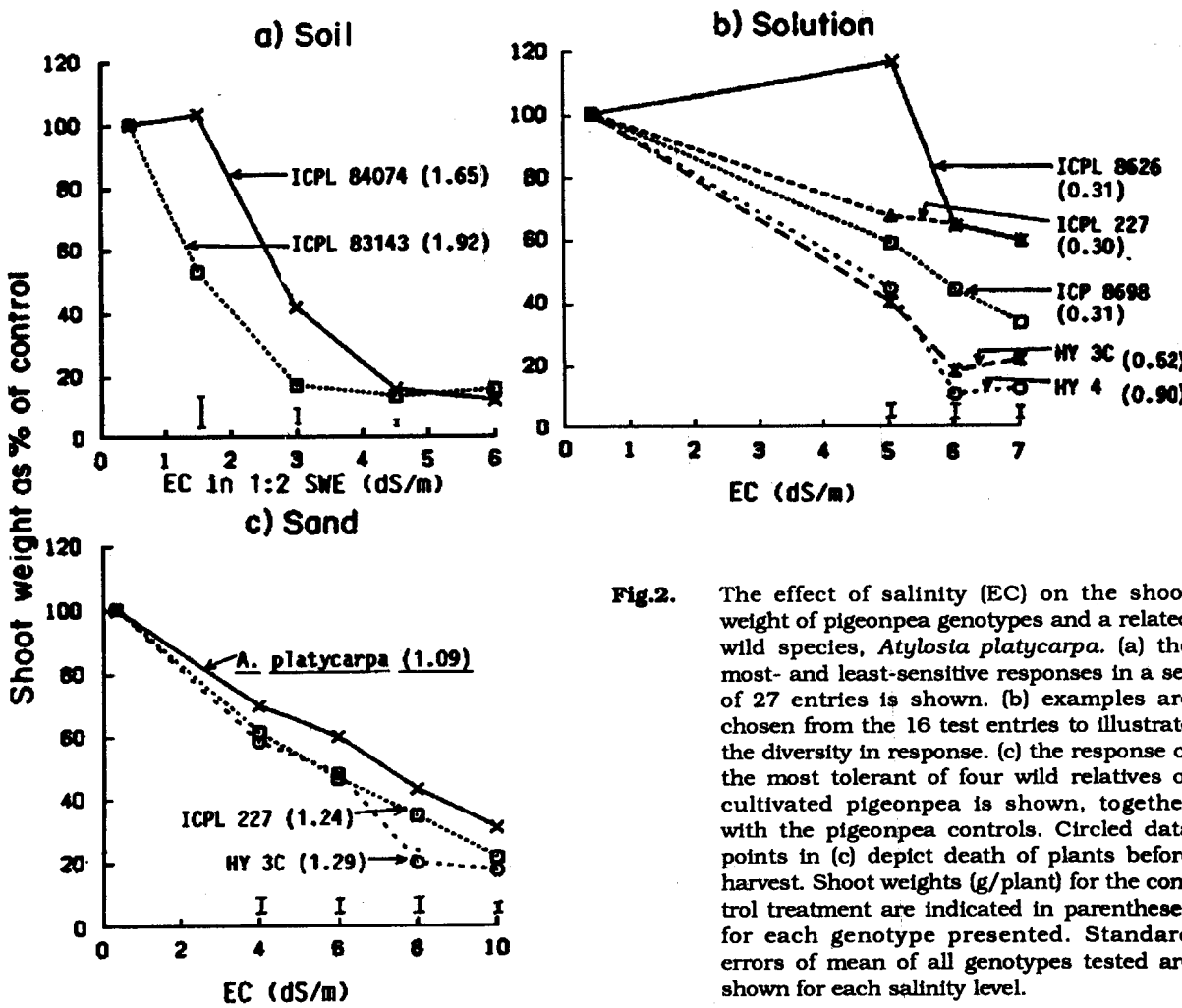

Fig.2. The effect of salinity (EC) on the shoot weight of pigeonpea genotypes and a related wild species, *Atylosia platycarpa*. (a) the most- and least-sensitive responses in a set of 27 entries is shown. (b) examples are chosen from the 16 test entries to illustrate the diversity in response. (c) the response of the most tolerant of four wild relatives of cultivated pigeonpea is shown, together with the pigeonpea controls. Circled data points in (c) depict death of plants before harvest. Shoot weights (g/plant) for the control treatment are indicated in parentheses for each genotype presented. Standard errors of mean of all genotypes tested are shown for each salinity level.

types were used as tolerant and susceptible controls. These 2 genotypes were also included in the present potted soil experiment but, in this case, their responses to salinity were almost identical and intermediate between the responses of the genotypes shown in Fig. 2a. In growth rankings of pigeonpea genotypes in natural saline fields, Hy 3C is usually found to be relatively susceptible to salinity damage. *Atylosia platycarpa* proved to be the most tolerant of all wild species tested, in this (Fig. 2c) as well as in other experiments not reported here. It was least affected at moderate salinity levels and had a 50% growth reduction at >6 dS/m. This species was able to adequately grow, flower and set pods at 10 dS/m. All plants died at 12 dS/m. Another *Atylosia* species, *A. albicans* was also more tolerant than ICPL 227 (results of this study not presented here) but the other wild species tested (in this and other experiments) either responded similarly to cultivated pigeonpea or were more sensitive to salinity.

Discussion

Various methods have been used to compare salinity response among plant genotypes (Maas & Hoffman, 1977). Threshold levels of EC, above which there is a significant yield decline, do not seem appropriate for the present data as many data points above and below the threshold level would be required to accurately define this level for the purpose of genotypic comparisons. Further, both chickpea (Lauter & Munns, 1986) and pigeonpea (Keating & Fisher, 1985) seem to have a small or negligible threshold level. The slope of the regression of decline in plant growth against increasing salinity has also been advocated as a measure of relative sensitivity to salinity (Maas & Hoffman, 1977). Again, many data points would be required to accurately measure slope and usually, as for much of the data in this study, the salinity response is curvilinear. Thus, for chickpea and pigeonpea it was prefered to compare EC at a given level of yield reduction caused by salinity.

By any criterion, the extent of variation in salinity response among the chickpea genotypes tested is surprisingly small. In solution culture studies, Lauter & Munns (1986) also showed that, while genotypic differences in salinity response of chickpea can be consistently demonstrated, the extent of the differences are small and vary between experiments. Although a range of wild species related to chickpea show considerable differences in salinity response, the variation is more towards salt sensitivity. Thus to date it has not been possible to identify any source of substantial salinity tolerance for chickpea, enough to warrant incorporation in a breeding programme to enhance salinity tolerance.

Apart from the limited set of chickpea genotypes used in this study, only one particular screening method, artificially salinized soil in pots, was used. However, this method tests a particular growth stage of chickpea vulnerable to salinity, the vegetative growth stage of plants dependent on symbiotic nitrogen fixation. Germination and seedling emergence in chickpea is generally less sensitive to salinity than later vegetative growth (Kumar, 1985) and, although there are genotypic differences in germination and emergence they do not necessarily translate into tolerance at later growth stages (Kumar, 1985).

The large variation in critical EC for a given genotype between experiments, even under relatively controlled conditions, is disturbing. However, it is well known that changes in soil moisture and atmospheric humidity affect transpiration rate which in turn affects rate of salt accumulation and thus determines extent of salinity damage (Sinha & Singh, 1976).

At this stage the best prospects of identifying substantial sources of salinity tolerance for chickpea, are suggested to be by judicious screening of the germplasm and related wild species, particularly those that have evolved in natural saline areas. There is a need for caution in expecting that cell and tissue culture techniques and evolving recombinant DNA methods will produce salt tolerant crop plants (Epstein & Rains, 1987). This is primarily because of the inte-

grated whole plant response to salinity (Gorham *et al.*, 1985) and its possible multigenic control (Tal, 1985).

Among the 27 pigeonpea genotypes compared for salinity response in potted soil (Fig. 2a), the genotypic variation was not much more than obtained for chickpea. However, the solution culture experiment indicates substantial differences in salinity response between pigeonpea genotypes at EC > 5 dS/m. Such diversity among pigeonpea cultivars has also been reported by Paliwal & Maliwal (1973). Further, related wild species such as *A. platycarpa* display even greater salinity tolerance than the best of the pigeonpea genotypes, being able to grow and function normally at EC levels in the vicinity of 10 dS/m. This is a level of tolerance worth incorporating into cultivated pigeonpea. However, *A. platycarpa* is incompatible for direct hybridization with cultivated pigeonpea and bridging techniques seem necessary to transfer not only salinity tolerance but also several other desirable traits of this wild species (ICRISAT, 1987).

Although the salinity responses in the present sand culture study were obtained on nitrogen fed plants, it was confirmed that symbiotically dependent *A. platycarpa* behaves similarly to nitrogen fed plants at high salinity levels. However, further genotypic comparisons need to be made using cultivated pigeonpea and wild relatives that have evolved in saline habitats.

Thus, at this stage, prospects for genetic enhancement of salt tolerance in pigeonpea appear more promising than for chickpea. Substantial sources of salinity tolerance have been identified in pigeonpea and related wild species, but this is not the case for chickpea. Further, pigeonpea is normally grown during a rainy season when soil salinity levels are likely to be at their lowest due to leaching by rainfall. On the other hand, chickpea is normally grown on receding soil moisture, which would tend to increase salt concentrations in the soil solution. Thus, to permit crop growth on natural saline soils, considerable enhancement of salinity tolerance would be required for chickpea, which is a relatively salt sensitive legume (Laüchli, 1984; Lauter & Munns, 1986).

References

Chauhan, Y.S. (1987). Screening for tolerance to salinity and waterlogging: Case studies with pigeonpea and chickpea. In *Adaptation of Chickpea and Pigeonpea to Abiotic Stresses.* Proceedings of the Consultants' Workshop, 19-21 December 1984, ICRISAT Center, India, pp. 93-103. Patancheru, India: ICRISAT.

Epstein, E.(1985). Salt-tolerant crops: Origins, development, and prospects of the concept. *Plant and Soil* **89**, 187-198.

Epstein, E. & Rains, D.W. (1987). Advances in salt tolerance. *Plant and Soil* **99**, 17-29.

Gorham, J., Wyn Jones, R.G. & McDonnell, E. (1985). Some mechanisms of salt tolerance in crop plants. *Plant and Soil* **89**, 15-40.

ICRISAT (1987). *Annual Report 1986*, pp. 203-204. Patancheru, India: ICRISAT.

Keating, B.A. & Fisher, M.J. (1985). Comparative tolerance of tropical grain legumes to salinity. *Australian Journal of Agricultural Research* **36**, 373-383.

Kumar, D. (1985). Emergence, establishment and seed yield of chickpea as affected by sodicity. *Annals of Arid Zone* **24**, 334-340.

Laüchli,A.(1984). Salt exclusion: An adaptation of legumes for crops and pastures under saline conditions. In *Salinity Tolerance in Plants: Strategies for Crop Improvement* (ed. R.C. Staples), pp. 171-187. New York: John Wiley & Sons, Inc.

Lauter, D.J. & Munns, D.N. (1986). Salt resistance of chickpea genotypes in solutions salinized with NaCl or Na_2SO_4. *Plant and Soil* **95**, 271-279.

Maas, E.V. & Hoffman, G.J. (1977). Crop salt tolerance - Current assessment. *Journal of the Irrigation and Drainage Division. American Society of Chemical Engineers* **103**, 115-134.

Paliwal, K.V. & Maliwal, G.L. (1973). Salt tolerance of some arhar (*Cajanus indicus*) and cowpea (*Vigna cinencis*) varieties at germination and seedling stages. *Annals of Arid Zone* **12**, 135-142.

Richards, R.A. (1983). Should selection for yield in saline regions be made on saline or non-saline soils? *Euphytica* **32**, 431-438.

Sinha, B.K. & Singh, N.T. (1976). Salt distribution around roots under different transpiration rates. *Plant and Soil* **44**, 141-147.

Tal, M. (1985). Genetics of salt tolerance in higher plants: Theoretical and practical considerations. *Plant and Soil* **89**, 199-226.

Talati, R.P. (1941). Damaged lands in Deccan and their classification. *Indian Journal of Agricultural Sciences* **11**, 959-977.