Comparative Salinity Responses among Pigeonpea Genotypes and Their Wild Relatives

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ABSTRACT

Soil salinity can be a major constraint to pigeonpea (*Cajanus cajan* (L.) Millsp.) in the regions where it is predominantly grown. This study was conducted to assess the extent of genetic variation for salinity tolerance in the germplasm of pigeonpea and its wild relatives. Solution culture experiments in a greenhouse and controlled environment chamber were conducted to screen a range of cultivated pigeonpea genotypes for ability to germinate and grow up to 60 d under saline conditions. Several wild relatives of pigeonpea were screened for salinity response in a sand culture system in a greenhouse. Among cultivated pigeonpea genotypes, ICPL 227 was one of the most tolerant and HY 3C one of the most sensitive genotypes tested. None of the pigeonpea genotypes tested were able to survive beyond 30 d at 8 dS m^{-1} or higher salinity levels. The extent of variation in salinity response among cultivated pigeonpea genotypes appeared too limited to warrant genetic enhancement of salinity tolerance. Among the wild relatives of pigeonpea, various species of *Atylosia*, *Rynchosia*, and *Dunbaria* showed a wide range of variation in their salinity tolerance (critical levels from 4 to 12 dS m^{-1}): *A. albicans* (W. & A.) Benth., and *A. platycarpa* could grow in a sand culture system at 12 dS m^{-1} and *Rynchosia albiflora* could not tolerate salinity levels above 4 dS m^{-1}. These results suggest that using wild relatives for genetic improvement may increase salinity tolerance of pigeonpea.

S ALINITY IN SOIL or water is of increasing importance to agriculture because it causes a stress condition to crop plants. Carter (1975) reported that more than one-third of the world's irrigated area is affected by salinity. In India alone, where 90% of the world's pigeonpea is produced, an estimated 12 million ha of land has been affected by salinity or alkalinity (Sharma and Gupta, 1986). It is often suggested that genetic enhancement of salinity tolerance should supplement traditional methods of reclamation and drainage as part of an integrated approach towards economic utilization of saline soils.

Genetic variation for salinity tolerance has been reported for pigeonpea and most crop species (Paliwal and Maliwal, 1973; Gururajarao et al., 1981; Keating and Fisher, 1985; Chauhan, 1987), but the extent and range of variation within a crop species generally seems to be limited. This is probably because most cultivated crop species have been selected in nonstress environments where salt tolerance traits, if any, would have been gradually lost from gene pools (Mudie, 1974; Maas and Nieman, 1978). If this hypothesis is correct, wild relatives of the crop species, which have not passed through the rigor of human selection, may provide a better chance of obtaining higher levels of tolerance to salinity.

Introduction of genes from the wild, salt-tolerant species can be used to enrich the gene pools of crop species (Tal, 1985). The lack of variation for salinity tolerance in the cultivated tomato [*Lycopersicon esculentum* (L.) Mill.] can be overcome by making wide crosses with related species (*L. cheesmanii* Riley and *Solanum pennellii*) that are highly salt tolerant (Tal and Shannon, 1983). The higher level of salinity tolerance of *Elytrigia elongata* (Host) Nevski, a wild wheat grass, was successfully transferred to the cultivated wheat (*Triticum aestivum* L.) and was expressed in the amphidiploids (Dvorak and Ross, 1986).

The extent of genetic diversity for salinity tolerance in pigeonpea germplasm has not been thoroughly explored. There are several wild relatives of pigeonpea belonging to the genera *Atylosia*, *Rynchosia*, *Dunbaria*, *Paracalyx*, *Eriosema*, and *Flemingia* (Remanandan et al., 1988), some of which can be crossed with the cultivated pigeonpea, but there is no information about their salinity tolerance. The objective of this investigation was to assess the exploitable genetic variation for salinity tolerance in germplasm of pigeonpea and its wild relatives.

MATERIALS AND METHODS

Experiment 1

One hundred and fifty pigeonpea genotypes were used in a preliminary screening study. These included germplasm lines from districts affected by salt in the Indian states of Uttar Pradesh, Punjab, and Maharashtra, as well as breed-

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ers' lines. Pigeonpea seeds were surface-sterilized with 20g kg^{-1} HgCl$_2$ solution for 5 min and then thoroughly washed with deionized water. The surface-sterilized seeds were sown in growth pouches (blotting paper envelopes within a polythene bag) by placing 10 seeds in the cleft of each pouch. The pouches were suspended in clear acrylic tanks (90 by 75 by 50 cm) and the bottom corners of the pouches were cut to expose a portion of the blotting paper to the nutrient solution in each tank. The outer surface of the tanks was painted black to prevent algal growth in the solution. A modified Arnon and Hoagland nutrient solution (0.25 strength) with 1.8 mM NH$_4$NO$_3$ was used as the growth medium. It was amended with NaCl + CACl$_2$ (1:1 w/w) for the 6 dS m^{-1} salinity treatment. This salinity level was determined as approximately critical for several pigeonpea genotypes in earlier studies (Subbarao, 1988). The control treatment, in which no salt was added to the nutrient solution, had an electrical conductivity (EC) of 0.36 dS m^{-1}. The composition of the nutrient solution was (mM): 0.23 KH$_2$PO$_4$, 0.52 KCl, 0.25 MgSO$_4$, 0.37 CaCl$_2$, 0.0015 MnSO$_4$, 0.00023 ZnSO$_4$, 0.00025 CuSO$_4$, 0.001 H$_3$BO$_3$, 0.00005 Na$_2$MoO$_4$, and 0.04 NaFeEDTA. A split-plot design with three replicates was employed. Greenhouse mean temperatures were 28/22 °C (day/night) and relative humidity 60 to 70% (mean day and night). Tank positions were rerandomized each week to minimize spatial effects in the greenhouse.

Each tank was supplied with 100 L of treatment solution at sowing. The nutrient solution was aerated throughout the experimental period. Solutions were monitored daily for EC and adjusted to the required level by adding an appropriate amount of deionized water. Treatment solutions were replaced every week. Tank tops were covered with black cotton cloth until 7 days after sowing (DAS) to ensure uniform seed germination and seedling emergence. Seedlings were thinned to five per pouch at 10 DAS, at which time germination counts were also made. Plants were allowed to grow until 60 DAS.

Experiment 2

Ten pigeonpea genotypes selected for tolerance or sensitivity in Exp. 1, were further examined at six salinity levels: 0, 5, 6, 7, 8, and 9 dS m^{-1}. The plant culture method was as described for Exp. 1, but one tank was used for each salinity level and four replicates of each genotype were included in each tank. The experiment was conducted in a controlled environment growth chamber where temperatures were 28/22 °C (day/night), relative humidity 60 to 70%, phytosynthetically active radiation (PAR) 100 to 120 W/m^{-2} and photoperiod 14 h. Plants were harvested at 50 DAS.

Experiment 3

Four wild relatives of pigeonpea, A. scarabaeoides, A. platycarpa, R. albiflora, and D. ferruginea, along with a tolerant (ICPL 227) and a sensitive (HY 3C) pigeonpea genotype, were selected for this study. To promote germination, seeds of these wild relatives were scarified by nicking the testa with a scalpel. Seeds were then surface-sterilized and germinated on water-absorbent blotting paper. The germination rolls were prepared from blotting papers (15 by 10 cm), in which seeds were centrally arranged and then rolled up. The rolls were then placed in plastic bags, moistened with distilled water and placed in an incubator at 28 °C.

The growth medium consisted of sieved river sand, washed and soaked in acid solution (pH 1–2) for 24 h, and then thoroughly washed with tap water. The sand was dried and placed in 18-cm-diam. polypropylene pots (2.5 kg sand pot^{-1}), which were subsequently steam sterilized. Seeds for each wild species were germinated before being sown, while

for cultivated pigeonpea the seeds were surface-sterilized, but not germinated. Each pot surface was covered with 50 g of polythene beads to minimize evaporation. Seedlings were thinned to four per pot at 10 DAS. The nutrient solution was as for Exp. 1, with salinity treatments of 0, 4, 6, 8, and 10 dS m^{-1} applied as NaCl + CaCl$_2$ (1:1 w/w). The experiment was conducted in a greenhouse, with four replicates in a randomized complete-block design, using conditions similar to those in Exp. 1.

Pots were irrigated with deionized water until 13 DAS. At 14 DAS, an initial salinity level of 4 dS m^{-1} was imposed by flushing each pot with 1L of 4 dS m^{-1} solution. To minimize salt shock to the seedlings for the 6 dS $^{-1}$ and higher salinity treatments, the initial salt level of 4 dS m^{-1} was gradually increased by 2 dS m^{-1} per day to the required levels. Nutrient solution alone was used for flushing the 0 salinity treatment (control). Every 4 d, pots were flushed with treatment solutions (250 mL pot^{-1}), to avoid salt build up. Pot positions were also randomized within each block every 4 d and evapotranspirational losses compensated daily with deionized water. Plants were harvested at 55 DAS. Shoots and roots were dried at 70 °C for 48 h and dry weights recorded.

Experiment 4

Ten species of Atylosia (A. albicans, A. goensis, A. acutifolia, A. cajanifolia, A. grandifolia, A. lineata, A. lanceolata, A. reticulata, A. sericea, and A. volubilis) were grown for 55 d at salinity levels of 0, 4, 6, 8, and 10 dS m^{-1} following the experimental details and growth conditions described for Exp. 3.

Statistics

Data were subjected to analysis of variance and least significant differences at $P = 0.05$ for the interaction term are quoted in the tables.

RESULTS AND DISCUSSION

Genetic variation in salinity response is a prerequisite for improving crop salinity tolerance (Shannon, 1985). In pigeonpea, there were significant differences among genotypes in their ability to germinate at a 6 dS m^{-1} salinity level. Among 150 genotypes tested (data presented only for selected genotypes), germination in the saline medium was as good as in the control (non-saline) in ICP 8695, ICP 11878, ICPL 228, ICPL 329, and ICP 8007 (Table 1), whereas ICP 8705 showed the maximum reduction (28%) at 6 dS m^{-1}. Genotypic differences in pigeonpea for germination under saline conditions have also been reported by Paliwal and Maliwal (1973) and Gururajarao et al. (1981). At germination, pigeonpea appears to be relatively more tolerant to salinity than at later growth stages. At 8 and 9 dS m^{-1}, none of the tested genotypes could survive beyond 4 wk (data not shown), even though germination was ≈60 to 70% in most genotypes (Table 2).

There was variation among pigeonpea genotypes in survival, growth, and leaf necrosis at 6 dS m^{-1} (Table 1). In genotypes ICP 8695, ICP 8594, ICP 8659, and ICP 11876, survival was as good as in the control. There was >70% mortality in some other genotypes, and 90% mortality was observed in ICP 8663. A breeders line, ICPL 227, produced dry matter equivalent to 71% of its control, without leaf necrosis, and

showed 95% survival. In contrast, pigeonpea geno-
types ICP 11795, ICP 11796, ICP 8663, and HY 3C
were the most adversely affected at 6 dS m^{-1}; the
growth reduction in them was >80% and mortality
>60%. The plants also showed severe leaf necrosis
(Table 1).

Most genotypic variation for salinity tolerance in
pigeonpea appeared to be confined to a narrow range
of salinity levels, between 5 and 7 dS m^{-1} (Tables 3
and 4). There were no substantial differences in
growth, survival, and leaf necrosis status between the
most-tolerant (ICPL 227, ICP 8594, ICP 8659, ICP
10103, and ICPL 112) and the least-tolerant (HY 3C,
ICP 8663, ICP 3783, ICP 9080, and ICP 11772) geno-
types at 5 dS m^{-1} (Tables 3 and 4). None of the most-
tolerant pigeonpea genotypes were able to survive at
≥8 dS m^{-1} in the hydroponic system. At 7 dS m^{-1}
these tolerant genotypes suffered a growth reduction
of ≈50%. In many of the sensitive genotypes the
growth was about 50% of the control at 5 dS m^{-1}.
Keating and Fisher (1985) observed a 50% growth re-
duction in two pigeonpea varieties within a range of
4.9 to 5.4 dS m^{-1}.

Genotypic variation of this magnitude, which is
confined to a narrow range of salinity levels, may not
be sufficient for genetic improvement of salinity tol-
erance in pigeonpea. Field salinity is a dynamic phe-
nomenon in which salt concentrations may change
across time and space, and genotypes selected for sa-
line conditions should perform reasonably well across
a range of salinity levels. In the present study, only
150 genotypes out of >10 000 germplasm lines avail-
able in pigeonpea (Remanandan et al., 1988) were
evaluated. The chances of obtaining a wider range of
variation in salinity tolerance may still exist. Salinity
tolerance has been increased through induced muta-
tions (Langridge, 1958; Ashraf et al., 1987), but this
method has its limitations (Nilan et al., 1969).

Various species of *Atylosia, Rynchosia,* and *Dun-
baria* showed a wide range of variation in their salinity
tolerance, from 4 to 12 dS m^{-1} (Tables 5 and 6). *Aty-
losia platycarpa* and *A. albicans* were shown in pre-
vious experiments (Subbarao, 1988) to tolerate and
grow at salt concentrations of 12 dS m^{-1}. *Atylosia pla-
tycarpa* was able to flower and produce pods at all
salinity levels, including 12 dS m^{-1} in this study. *Ryn-
chosia albiflora* was at the other extreme, being able
to withstand salinity levels only ≤4 dS m^{-1}.

Table 2. Effect of different levels of salinity on germination of se-
lected pigeonpea genotypes in solution culture (Exp. 2).

Genotype	Salinity level (dS m^{-1})
	0	5	6	7	8	9
	%
ICP 8594	85	85 (100)†	75 (88)	75 (88)	70 (82)	70 (82)
ICP 8659	90	78 (87)	75 (83)	70 (78)	65 (72)	60 (67)
ICP 10103	95	85 (89)	73 (77)	75 (79)	70 (74)	65 (68)
ICPL 112	95	83 (87)	75 (79)	70 (74)	65 (68)	60 (63)
ICPL 227	90	85 (94)	85 (94)	75 (83)	70 (78)	70 (78)
ICP 8663	80	75 (94)	70 (88)	63 (79)	60 (75)	55 (69)
ICP 3783	85	70 (82)	63 (74)	58 (68)	50 (59)	40 (47)
ICP 9080	90	78 (87)	75 (83)	70 (78)	65 (72)	60 (67)
ICP 11772	90	80 (89)	70 (78)	65 (72)	60 (67)	60 (67)
HY 3C	95	80 (84)	80 (84)	70 (74)	65 (68)	60 (63)
LSD (0.05) = 10.5						

† Figures in parentheses are percentages of control.

Table 3. Effect of different levels of salinity on the survival and leaf
damage (LD: yes = necrosis present, no = necrosis absent) of
selected pigeonpea genotypes at 50 DAS (Exp. 2).

Genotype	Salinity level (dS m^{-1})†
	\multicolumn{2}{6}	\multicolumn{2}{7}
	Survival	LD	Survival	LD
	%		%	
ICP 8594	100	no	90	no
ICP 8659	93	no	93	no
ICP 10103	95	no	90	no
ICPL 112	80	no	70	no
ICPL 227	100	no	93	no
ICP 8663	30	yes	10	yes
ICP 3783	40	yes	20	yes
ICP 9080	15	yes	10	yes
ICP 11772	35	yes	20	yes
HY 3C	30	yes	20.	yes
LSD (0.05) = 7				

† All genotypes had 100% survival and no leaf damage at 0 and 5 dS m^{-1},
but none survived at 8 and 9 dS m^{-1} beyond 30 days after sowing.

Table 1. Effect of salinity on germination, survival, growth and leaf
damage (LD: yes = necrosis present, no = necrosis absent) of
selected pigeonpea genotypes in solution culture (Exp. 1).

Genotype	Germination at 6 dS m^{-1}	Survival at 6 dS m^{-1}	\multicolumn{2}{Shoot dry matter (mg plant^{-1})}	LD
			Control	6 dS m^{-1}	
	%	%			
ICP 8695	80 (100)†	100	258	132 (51)†	yes
ICP 8594	75 (90)	100	659	377 (58)	no
ICP 8659	77 (93)	100	454	240 (58)	no
ICP 11876	73 (78)	100	287	165 (57)	no
ICPL 227	85 (94)	95	352	256 (71)	no
ICP 10103	73 (78)	86	390	240 (61)	no
ICPL 112	80 (92)	80	567	287 (51)	no
ICP 11878	77 (100)	76	327	170 (52)	yes
ICPL 228	77 (100)	86	303	121 (40)	no
ICPL 329	83 (100)	80	290	121 (42)	yes
ICP 8007	80 (100)	46	185	42 (22)	yes
ICP 8705	63 (72)	46	267	60 (23)	yes
ICP 11795	75 (83)	40	254	36 (15)	yes
ICP 11772	80 (82)	35	288	60 (21)	yes
ICP 3783	80 (89)	30	450	90 (20)	yes
ICP 11796	70 (80)	26	303	46 (15)	yes
ICP 9080	77 (89)	26	217	63 (29)	yes
HY 3C	72 (88)	25	351	55 (16)	yes
ICP 8663	67 (92)	10	373	58 (16)	yes
LSD (0.05)	11	6	37 (7.9)		

† Figures in parentheses are percentages of control.

Table 4. Effect of salinity on shoot dry matter of selected pigeonpea
genotypes in solution culture at 50 DAS (Exp. 2).

Genotype	Salinity level (dS m^{-1})
	0	5	6	7
	mg plant^{-1}
ICP 8594	725	439 (59)†	388 (53)	363 (50)
ICP 8659	955	575 (60)	525 (55)	459 (48)
ICP 10103	662	429 (63)	330 (50)	264 (40)
ICPL 112	741	446 (60)	355 (48)	312 (42)
ICPL 227	558	391 (70)	362 (65)	277 (50)
ICP 8663	544	326 (60)	162 (30)	96 (18)
ICP 3783	725	370 (51)	255 (35)	107 (15)
ICP 9080	600	300 (50)	180 (30)	31 (5)
ICP 11772	690	352 (51)	140 (20)	70 (10)
HY 3C	646	265 (41)	122 (19)	65 (10)
LSD (0.05) = 95				

† Figures in parentheses are percentages of control (LSD (0.05) = 7.6).

Table 5. Effect of salinity on the shoot dry matter of wild relatives of pigeonpea (*Atylosia, Rynchosia* and *Dunbaria* spp.), ICPL 227 and HY 3C at 55 DAS in sand culture (Exp. 3).

Species/genotype	Salinity level (dS m⁻¹)				
	0	4	6	8	10
	g pot⁻¹				
A. platycarpa	16.25	13.17 (81)†	12.35 (76)	10.08 (62)	8.45 (52)
A. scarabaeoides	1.09	0.77 (71)	0.56 (51)	0.30 (28)	0.11 (10)
R. albiflora	0.73	0.19 (26)	0.15 (21)	0.08 (12)	0.07 (9)
D. ferruginea	0.76	0.53 (70)	0.32 (42)	0.24 (31)	0.06 (8)
ICPL 227	4.11	2.88 (70)	2.47 (61)	1.64 (41)	0.41 (9)
HY 3C	4.11	2.30 (51)	1.23 (30)	0.65 (16)	0.21 (5)
LSD (0.05) = 0.33					

† Figures in parentheses are percentages of control (LSD (0.05) = 6.0).

Table 6. Response of shoot dry matter of *Atylosia* species to salinity at 55 DAS in sand culture (Exp. 4).

Atylosia species	Salinity level (dS m⁻¹)				
	0	4	6	8	10
	g pot⁻¹				
A. albicans	7.92	6.48 (82)†	5.85 (74)	5.14 (65)	3.81 (48)
A. sericea	9.31	7.54 (82)	6.24 (68)	5.49 (59)	4.00 (43)
A. cajanifolia	8.70	6.38 (75)	5.10 (60)	4.09 (48)	2.87 (34)
A. volubilis	10.13	7.20 (72)	6.28 (62)	5.37 (53)	2.53 (25)
A. reticulata	4.83	3.27 (68)	3.19 (67)	1.73 (37)	0.43 (9)
A. grandifolia	3.92	2.35 (59)	1.65 (43)	0.79 (21)	0.16 (4)
A. lineata	7.14	3.93 (56)	2.97 (42)	1.86 (26)	0.71 (10)
A. goensis	8.31	5.71 (69)	3.94 (48)	0.83 (10)	0.42 (5)
A. lanceolata	5.72	2.76 (48)	2.21 (39)	1.12 (20)	0.29 (5)
A. acutifolia	3.48	1.42 (41)	1.05 (30)	0.43 (12)	0.17 (5)
LSD (0.05) = 0.60					

† Figures in parentheses are percentages of control (LSD (0.05) = 8.5).

Among the 15 wild relatives tested, *A. platycarpa, A. albicans, A. sericea,* and *A. cajanifolia* were distinctly superior in their salinity tolerance compared with the most tolerant pigeonpea genotype ICPL 227. This certainly enhances the possibilities of increasing salinity tolerance of pigeonpea by introgressing the higher levels of tolerance from these wild relatives. *Atylosia platycarpa* is incompatible for direct hybridization with cultivated pigeonpea, and bridging techniques are necessary to transfer not only salinity tolerance but also other desirable traits of this species (Dundas et al., 1989); however, *A. albicans,* which has the same level of tolerance as *A. platycarpa,* is compatible with the cultivated pigeonpea and the higher level of tolerance could thus be directly transferred to pigeonpea. The tolerance in *A. sericea* and *A. cajanifolia* is similarly transferable to pigeonpea. The remaining wild relatives (*A. scarabaeoides, A. volubilis, A. reticulata, A. grandifolia, A. lineata, A. goensis, A. lanceolata, A. acutifolia, D. ferruginea,* and *R. albiflora*) are either similar or inferior to ICPL 227 in their salinity tolerance; however, this study is based on only one accession from each species. Considering the large number of accessions available within each species

(Remanandan et al., 1988), even higher levels of salinity tolerance than what has so far been identified in *A. platycarpa* and *A. albicans* are possible. This could be realized through systematic evaluation of all accessions in wild species showing salinity tolerance.

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