Screening for intra and inter specific variability for salinity tolerance in Pigeonpea (*Cajanus cajan*) and its related wild species

Namita Srivastava, V.Vadez*, HD Upadhyaya, and K.B. Saxena

*** Correspondence**

Introduction

Pigeonpea is one of the major legume crops grown in the semi arid tropics, particularly in India. Its high sensitivity to salinity poses a major constraint to crop production in certain areas (Chauhan, 1987). Salinity is an ever increasing abiotic stress to the cultivated land, which affects plant growth, development and yield. Worldwide, approximately 100 million ha of arable land are affected by salinity, which accounts for about 6-7% of total (Munns and James, 2003). In India, where 90 % of world's pigeonpea is produced around 13.3 million ha land is affected by salinity (Consortium for Unfavorable Rice Environment, IRRI, 2003).

Besides expensive management options to prevent and remediate salinity, such as proper irrigation practices, reclamation of saline soil, leaching of salts from soil profile by efficient drainage, introduction of salinity tolerant varieties in such areas could partly ease the limitation. However, little efforts have been made to breed for salinity tolerance in economically important crops (Flowers, 2004), although there is more and more knowledge available about the genes involved in salinity response and tolerance in model crops like arabidopsis and rice. Salt tolerance is a complex phenomenon that induces morphological and developmental changes, involving physiological and biochemical processes. For instance in legumes, salt strongly affects N_2 fixation (Rao et. al. 2002). No information is available on the nature of salinity tolerance in pigeonpea, except an earlier work to screen pigeonpea for salinity tolerance (Dua and Sharma, 1996). No attempt has been made to breed tolerant lines, besides an early and discontinued effort by Subbarao et al. (1990). Therefore, a study of the genetic variation in salinity responses would be the first step before undertaking breeding efforts (Shannon, 1985).

Therefore, the objectives of this work were to: (i) standardize a screening technique for salinity tolerance at vegetative stage, (ii) assess the genetic variation for salinity tolerance by the relative biomass production under saline conditions, in the minicore collection of pigeonpea (Upadhyaya et.al. 2006), in wild relatives, and in germplasm originating from

areas putatively affected by salinity, and (iii) observe the shoot Na^+ concentration and its relation with salinity tolerance.

Materials and methods

Standardization of an adequate dose for salinity screening

To assess the most suitable salt treatment for screening, two experiments were carried out in a glasshouse, with day/night temperature of 28/22 ºC. In both experiments, six pigeonpea genotypes of different maturity groups (ICPL 88039, ICPL 88034, ICPL 87119, ICPL 96058, ICP 7035 and ICPL 366) were grown in 6" pots filled with 2.0 kg of Alfisol, collected from the experimental station at ICRISAT. The soil was fertilized with diammonium phosphate (DAP) at 300 mg kg^{-1} soil. Four seeds were planted per pot and later thinned to two seedlings per pot. Five replicated pots per treatment and genotype were grown. In both the experiments, NaCl was applied at a fixed rate in g kg^{-1} of soil. In Experiment 1 (Exp.1), treatments were 0, 1.34, 2.68, 4.02 g NaCl pot^{-1}, whereas 0, 1.34, 2.01, and 2.68 g pot^{-1} were used in Experiment 2 (Exp.2). Treatments were applied as three split doses within the first 10 days after sowing to avoid a too rapid build up of salt in soil in Exp. 1 and one application at the time of sowing in Exp.2. Plants were grown for seven weeks in both the experiments and harvested. At harvest, plants were separated into leaves, stems, pods, and nodulated roots and oven dried for three days at 70 ^{0}C. Since pod weight was negligible in different saline treatments, it was included in the shoot biomass.

In both experiments, plants were little affected by a treatment of 1.34 g NaCl pot^{-1} for which biomass was up to 79% of control. On the contrary, plants were severely affected by 2.68 g NaCl pot^{-1} and 4.02 g NaCl pot^{-1} for which biomass produced was respectively only 26 and 6% of control in Exp.1. There was a decent growth with a treatment of 2.01 g NaCl pot^{-1} and biomass produced was 41% of control in Exp.2. This latter treatment appeared to be adequate because it was neither too severe, nor too mild. Genotypic differences were also the largest at this rate of salt application (data not shown) (Srivastava et al. 2005). We used this treatment (equivalent to 1.01 kg^{-1}) to screen a large number of genotypes.

Screening of 286 genotypes

Using the standardized treatment of 1.01g NaCl kg^{-1} soil, a large set of pigeonpea genotypes were screened including 150 genotypes of the recently established minicore collection, 68 different accessions from seven wild relatives of pigeonpea, 68 accessions collected from putative saline areas of Bangladesh, Taiwan, Ethiopia, Indonesia, Argentina, Iran, and Brazil. The experiment was planted on 31 July, 2005 in outdoor conditions equipped with a rainout shelter to prevent rain. Experimental design was an alpha lattice (20x15) with three replications. The experiment was planted in 8'' pots, filled with 5 kg of Alfisol, collected from the experimental station at ICRISAT, similar the soil used in the preliminary experiments. The trial had two treatments i.e. control (irrigated with soft water) and saline (5.04 g pot^{-1} applied in three doses of 1.68 g pot^{-1} each time). The first split dose was diluted in sufficient amount of water to saturate the soil profile. The second and third doses were dissolved in the amount of water needed to almost re-saturate the soil profile. The field capacity of pots was maintained throughout the experiment.

The experiment was harvested at 69 DAS on 8 October, 2005. At the time of harvest plants were separated into leaves and stems. There was little flowering in short duration genotypes, and pods, if any, were included in the shoot biomass. The shoot biomass for each sample was analyzed using the statistical procedure of residual maximum likelihood (ReML) by treating the replication and replication x block effect as fixed for the best linear predictions (BLUPs) for the performances of the 286 genotypes. To assess salinity tolerance, the percent relative reduction under saline conditions compare to control (RR %) was computed as

$$\mathbf{RR\% = 1 - (biomass\ under\ salinity/biomass\ under\ control)}$$

and the salinity susceptibility index (SSI), as

$$\mathbf{SSI = (1-Y_{SS}/Y_{NS})/SII}$$

Where Y_{SS} and Y_{NS} are the mean biomass of a given accession in saline and non-saline conditions respectively and SII is the salinity intensity index, calculated as

$$\mathbf{SII=1-X_{SS}/X_{NS}}$$

Where X_{SS} and $X_{NS,}$ are the means of all accessions under salinity stressed and non-stressed environments respectively (Fisher and Maurer, 1978). Therefore, SSI provides

an assessment of the relative performance of a given entry with regard to the mean performance of all the entries. On the basis of SSI and RR% data for biomass under salinity compare to control, the group mean was calculated for each set of materials. The genotypes with SSI and RR% value below the group mean minus one standard deviation were considered highly tolerant and with the SSI and RR% value above the group mean plus one standard deviation as highly sensitive. This approach was used to assess the level of tolerance and susceptibility within each and across the groups of genotypes included in that screening.

Na^+ concentration in shoot

In Exp.1 and in the screening of 286 genotypes, 150 mg of finely ground shoot samples were digested in 4 ml of concentrated sulphuric acid with 0.5% selenium powder at 360^0C for 75 min on a block digester and the digest was diluted to 75 ml using distilled water. This dilution was used to estimate Na^+ (Sahrawat et. al. 2002) using an atomic absorption spectrophotometer (Varion model 1200, Australia).

Results and discussion

Performance of 286 pigeonpea genotypes for salinity tolerance

The mean RR% and SSI across groups of genotypes (Wild, selected accessions, and minicore collection) were not very different from one group to another, showing that no group had any particular tolerance or sensitivity compare to the others (Table 1). Therefore, we looked at the range of variation for RR% and SSI within each of these groups of genotypes.

Wild species

Sixty-eight different accessions of seven different wild species *viz C.acutifolius, C. cajanifolius, C.lineata, C. lanceolata, C. platycarpus, C.scarabaeoides,* and *C. sericea* were studied. In this group, genotypes having SSI values > 1.44 and RR% > 96% were considered as sensitive, whereas the genotypes with SSI < 0.62 and RR < 41% were classified as tolerant (Table 2) (group mean SSI or RR% plus and minus one standard deviation).

To assess the relative tolerance or susceptibility of given species, we analyzed the median SSI values across wild species. A high SSI and RR% median value in a specie would indicate that more than half of the genotypes of that specie are sensitive and that the

specie can overall be considered as sensitive. Among the 12 accessions of *C.acutifolius* studied, ICPW 1 and ICPW 10 were highly tolerant whereas seven genotypes were highly sensitive (Table 2). Based on estimated median for SSI (1.5) *C. acutifolius* collection tended to be more sensitive. None of the *C. cajanifolius* and *C. lineata* accessions showed salinity tolerance. In fact, the median SSI of *C. cajanifolius* was very high (1.27) (Table 3). Among the 13 accessions of *C. platycarpus* tested, three (ICPW 66, ICPW 67 and ICPW 68) were tolerant and originated from Maharastra and Uttar Pradesh. Of the 24 accessions of *C. scarabaeoides* nine were sensitive whereas seven were highly tolerant (Table 2). This is an interesting finding, which opens the possibility to develop mapping populations from *C. scarabaeoides*, where there is more probability to find genetic polymorphism between the parents than in the cultivated germplasm, and therefore chances to identify QTL more rapidly than from crosses involving cultivated materials. Median value for SSI in *C. scarabaeoides* was 0.89, showing that a majority of accessions in this species were relatively tolerant (Table 3). Among the wild species *C. scarabaeoides* exhibited genotypic differences for SSI and RR% and all of them originated from different parts of India except ICPW 94, from Sri Lanka. Among the four accessions of *C. sericea* two (ICPW 160, and ICPW 161) were salinity tolerant and from Maharastra and West Bangal respectively. Hence three species *viz* C. *platycarpus*, *C. scarabaeoides* and *C. sericea* manifested relatively more tolerance to salinity with median values for SSI of 0.96, 0.89, and 0.60 respectively (Table 3).

Considering all the wild species accessions, ICPW 87, ICPW 94 and ICPW161 were the most tolerant to salinity, having SSI values as low as 0.03, 0.28 and 0.13 and RR% values as low as 1.97, 18.65 and 8.33. These were from *C. scarabaeoides* and *C. sericea*, respectively. One accession of *C. platycarpus*, ICPW 68, was also found very tolerant, having low SSI and RR% values (0.37 and 24).

Selected landraces from saline areas

For the accessions selected from different putatively saline areas for the cultivation of pigeonpea, the genotypes with higher SSI than 1.27 and RR% higher than 84 were considered as salinity sensitive accessions whereas genotypes with SSI value lesser than 0.87 and RR% values less than 57 were considered as salinity tolerant genotypes (Table 2). Thirteen accessions were identified as tolerant (RR% ranged between 42 and 55 and

SSI ranged between 0.64 and 0.84) (Table 2). All the tolerant genotypes of pigeonpea in this group originated either from Bangladesh or Indonesia. Eleven genotypes were found sensitive with RR% ranging between 85 and 100 and SSI values between 1.29 and 1.52 (Table 2). These originated from Bangladesh, Brazil, Ethiopia and Indonesia. The estimates of SSI median for this group (1.09) revealed low frequency for tolerance (Table 3).

Minicore collection

In case of minicore collection of pigeonpea, genotypes were classified as tolerant with SSI values lesser than 0.80 and RR% lesser than 53 whereas genotypes were identified as sensitive when having SSI values above 1.18 and RR% values above 78 (Table 2). In this group, RR% ranged from 15 to 53 and SSI from 0.23 to 0.80 for tolerant genotypes. Among the 150 genotypes of the minicore collection, 16 were considered as tolerant. These are ICP 8860, ICP 7803, ICP 7260, ICP 6815, ICP 10654, ICP 3046, ICP 2746, ICP 7426, ICP 10559, ICP 7057, ICP 6049, ICP 6859, ICP 7, ICP 14722, ICP 11477, and ICP 6128 and originated from India and Bangladesh. Twenty five genotypes were classified as sensitive with a range of RR% between 78 and 100 and a SSI range between 1.18 and 1.52 (Table 3). These genotypes originated from India, Kenya, Malawi, Australia, Tanzania, Jamaica, and Venezuela.

The minicore of pigeonpea showed SSI median value 0.97, smaller than the SSI median value of 1.09 found for the selected accessions from saline areas. This indicated the level of tolerance was relatively higher in the minicore collection than in those accessions putatively selected from salinity affected areas (Table 3).

Na^+ accumulation in shoot and salinity tolerance

In most plants, the accumulation of Na^+ in shoot brings about deleterious effects and plant strategy is to limit the Na^+ build up in shoot tissues. In Exp. 1, Na^+ concentration in shoot also increased with the increase of salt concentration and ICPL 88039, which had the highest shoot biomass across salt treatments showed the least Na^+ accumulation compare to other genotypes (data not shown). In fact, there was a negative significant relationship ($r=0.78$, $P>0.001$) between shoot Na^+ accumulation and the ratio of biomass, our proxy for salinity tolerance in the 2.68 g NaCl pot^{-1} treatment (fig 1a). We found a similar relation with the landraces collected from different saline prone areas worldwide,

which also showed a negative and significant correlation (r=0.72, P>0.01) between the ratio biomass and Na^+ accumulation (fig 1c). In case of the minicore collection, the ratio of biomass and Na^+ accumulation also showed a negative and significant correlation (r=0.51, P>0.01) (fig.1d). Such relation was not found in the group of wild accession, where there was a negative and non significant (r=0.36) correlation between the ratio of biomass and the total Na^+ accumulation in the shoot (fig 1b).

Conclusion

We found that a NaCl treatment of 1.01 g kg^{-1} Alfisol was suitable to salinity screening in pigeonpea. Using that treatment, we found large variation in the SSI and the RR% in both cultivated and wild accessions. The amount of Na accumulation in shoot showed that more tolerant materials accumulated less Na in shoot (Fig. 1) except the wild species. following a different pattern than cultivars i.e. negative but non significant correlation between the ratio biomass and Na^+ accumulation.

Overall, we found that wild species *C. acutifolius, C.cajanifolius* and *C.lineata* were mostly sensitive, whereas *C. platycarpus, C. scaraboides* and *C. sericea* provided good sources of tolerance. It was interesting to notice that *C. scaraboides* also provided a large range of sensitive materials. Although we would have expected that accessions originating from putative saline areas would provide higher levels of tolerance, the minicore collection of pigeonpea provided a larger range of variation in the salinity response. It should be noted that, either from the minicore collections, or the set of accessions from putatively salinity affected areas, there was a large number of tolerant accessions originating from Bangladesh. Further work is on going to confirm these data, to assess yield response to salinity, and to develop intra-or inter-specific populations for the mapping of salinity tolerance.

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Table 1: Total group means of wild, selected accessions, and minicore for the salinity susceptibility index (SSI) and the relative reduction percentage (RR%) at 5.04 g pot^{-1} NaCl compare to control at ICRISAT, Patancheru, India in October 2005.

Groups	Mean± SD
Wild	
SSI	1.03 ± 0.41
RR%	68.7 ± 27.6
Selected accessions	
SSI	1.07 ± 0.2
RR%	70.6 ± 13.6
Minicore	
SSI	0.99 ± 0.19
RR%	65.3 ± 12.8

Table 2: Pigeonpea tolerant and sensitive accessions grouped by level of salinity tolerance (Tolerant and Sensitive) based on salinity susceptibility index (SSI) and percent relative reduction, assessed under control conditions and in 5.04 g pot^{-1} NaCl at ICRISAT, Patancheru, India in October 2005.

	Tolerant			Sensitive		
Species	**Accessions**	**Range (SSI)**	**Range (RR)**	**Accessions**	**Range (SSI)**	**Range (RR)**
C. acutifolius	ICPW 1, and ICPW 10	0.41-0.59	27-39	ICPW 3, ICPW 4,ICPW 5, ICPW 6, ICPW 7, ICPW 8,and ICPW 9	1.5	100
C.cajanifolius	*	*	*	ICPW 28	1.5	100
C.lineata	*	*	*	ICPW 44,ICPW 47,and ICPW 48	1.5	100
C.lanceolata	*	*	*	*	*	*
C.platycarpus	ICPW 66, ICPW 67,and ICPW 68	0.37-0.60	24-40	*	*	*
C. scarabaeoides	ICPW 87,ICPW 94, ICPW 132, ICPW 130, ICPW 126, ICPW 117, ICPW 129, and ICPW 125	0.03-0.61	2.0-41	ICPW 91, ICPW 96, ICPW 97,ICPW 98, ICPW 99, ICPW 100, ICPW 101, ICPW 102, ICPW 123 and ICPW 124	1.5	100
C. sericea	ICPW 160, and ICPW 161	0.13-0.48	8-32	ICPW 159	1.5	100
Selected accessions	ICP 13991, ICP 14974, ICP 13997, ICP11412, ICP 11413, ICP11419, ICP 11425, ICP 11435, ICP 11426, ICP 11418, ICP 14973, ICP 11432, and ICP 11424,	0.64-0.84	42-55	ICP 11414, ICP 11420, 1CP 14175, ICP 13996, ICP 13625, ICP 13550, ICP 13629, 1CP 11427, ICP 14865, ICP 11434, and ICP 14972	1.29-1.52	85-100
Minicore	ICP 8860, ICP 7803, ICP 7260, ICP 6815, ICP 10654, ICP 3046, ICP 2746, ICP 7426, ICP 10559, ICP 7057, ICP 6049, ICP 6859, ICP 7, ICP 14722, ICP 11477, and ICP 6128	0.23-0.79	15-52	ICP 1071,ICP 6739,ICP15382,ICP 15493,ICP 8793,ICP 13139,ICP14155,ICP 13431, ICP14368,ICP 11910,ICP 13191,ICP 15161,ICP 9336,ICP 15185,ICP 3576,ICP 13359,ICP 1273,ICP 12123,ICP 6992,ICP 8863,ICP 121.5,ICP 14120,ICP 14094, and ICP 15109,	1.18-1.52	78-100

Table 3: Range of variation in the different species of wild pigeonpea for the salinity susceptibility index (SSI) and the percent relative reduction (RR%), shoot biomass under saline (DW (S)) and control (DW (C)), median SSI and median DW (S) under a treatment of 5.04 g pot^{-1} NaCl, for the groups of different pigeonpea accessions.

Wild Species	Number of accessions	Range SSI	Range RR%	Range DW (S)	Range DW (C)	Median SSI	Median DW (S)
(1) C.acutifolius	12	0.41-1.52	27-100	0.00-3.63	2.29-14.37	1.5	0
(2) C. cajanifolius	4	0.72-1.52	47-100	0.00-10.22	15.07-19.37	1.27	2.75
(3) C. lineata	10	0.72-1.52	48-100	0.00-4.95	6.29-13.08	1.25	1.68
(4) C. lanceolata	1	1.35	89	0.61	5.65	1.34	0.61
(5) C. platycarpus	13	0.37-1.40	24-92	0.61-6.20	8.02-15.78	0.96	3.93
(6) C. scarabaeoides	24	0.03-1.52	2-100	0.00-11.29	5.56-15.45	0.89	5.39
(7) C.sericea	4	0.13-1.52	8-100	0.00-8.09	8.10-12.34	0.6	6.85
Selected accessions	68	0.64-1.52	42-100	0.00-13.37	9.35-29.61	1.09	7.02
Minicore	(146 lines+4 checks)	0.23-1.52	15-100	0.00-13.34	8.41-28.70	0.97	7.84

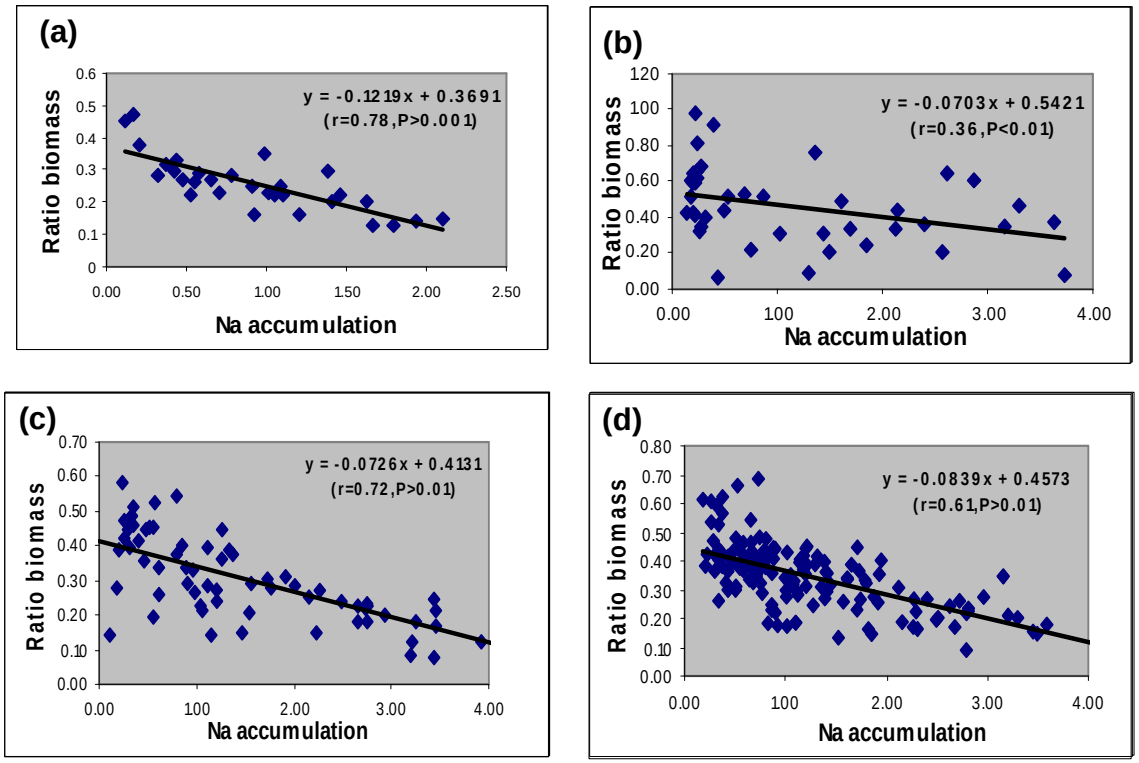

Figure 1. Simple linear correlation between the ratio of biomass (biomass under salinity divided by biomass under control) and Na^+ accumulation in shoot: (a) with a treatment of 1.34 g NaCl kg^{-1} soil in six genotypes of different maturity group, (b) in wild species (c) in selected landraces from saline areas (d) in the minicore collection. Data are the mean of 5, 3, 3, and 3 replicated data of each genotype, for (a), (b), (c) and (d) respectively.