Screening Chickpea Mini-core Germplasm for Tolerance to Soil Salinity

R Serraj, L Krishnamurthy and **HD Upadhyaya**
(ICRISAT, Patancheru 502 324, Andhra Pradesh, India)

Chickpea (*Cicer arietinum*) is generally grown in the semi-arid regions where soil salinity is one of the major constraints for yield production (Rengasamy 2002). Extensive screening for salinity tolerance has been carried out under field conditions (Dua 1992) and subsequent recommendations of chickpea varieties suitable for cultivation in saline soils were made (Dua and Sharma 1995). However, most of these studies involved limited genetic base catering for narrow geographical region. To know the complete range of tolerance levels available in cultivated chickpea, it becomes necessary to evaluate the whole range of germplasm collection. The availability of a subset of the entire chickpea germplasm collection as mini-core collection (Upadhyaya and Ortiz 2001) provides access to evaluate a manageable number of accessions while capturing nearly the whole range of variation for responses to abiotic or biotic constraints limiting yield. Identification of larger number of salinity tolerant sources would also permit use of diverse sources for future breeding efforts and to ensure a better chance of success in improving the salinity adaptation of chickpea.

Evaluation of large number of accessions for yield responses to salinity under field conditions can be difficult due to the spatial and temporal variability. However, their pre-flowering stage response can be adequate for initial screening. Therefore, the main objectives of this study are to: (1) assess the extent of genetic variation available for salinity tolerance in the mini-core germplasm collection of chickpea at the vegetative stage of development; (2) identify accessions with contrasting salinity responses; and (3) assess the comparative level of tolerance available in chickpea breeding lines and popular varieties.

This screening was conducted in pots (24 cm diameter and 22 cm height, with 7 kg Vertisol) under open field conditions in an alpha lattice design (14 × 18) with three replications at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. The experiment was conducted between 19 December 2003 and 28 January 2004, with no rainfall events, at a minimum temperature of 8 to 19°C and maximum of 23 to 31°C. Chickpea mini-core germplasm accessions (211) and 41 popular varieties and breeding lines were grown in two salinity treatments: (1) Control: irrigated with tap water; and (2) Saline: irrigated with 100 mM NaCl solution to field capacity of the soil once at the time of sowing (resulting in EC of 1.7 dS m^{-1} of 1:2 soil:distilled water extract), and subsequently irrigated with tap water. Twelve seeds for each entry were sown on 19 December 2003 in four equally spaced hills in each pot and irrigated with tap water or saline solution to field

Table 1. Trial means, range of best linear unbiased predicted means and analysis of variance of shoot biomass under salinity and their ratio as that of control of 252 chickpea entries sampled at 15, 21, 28 and 40 days after sowing (DAS) at ICRISAT, Patancheru, India during 2003/04.

Trait	Trial mean	Range of predicted means	SEd	$\sigma^2_g \pm$ SE	CV (%)	Heritability in broad sense (h^2)
Shoot dry matter (g plant^{-1}) under 100 mM salinity						
15 DAS	0.061	0.029–0.133	0.0120	0.00053 ± 0.00005	24.8	0.698
21 DAS	0.111	0.073–0.268	0.0224	0.00153 ± 0.00016	25.8	0.652
28 DAS	0.173	0.082–0.371	0.0290	0.00476 ± 0.00047	20.5	0.792
40 DAS	0.309	0.117–0.935	0.0828	0.03158 ± 0.00317	33.0	0.752
Ratio of shoot dry matter under 100 mM salinity as that of control						
15 DAS	0.621	0.524–0.883	0.1209	0.01234 ± 0.00256	32.0	0.234
21 DAS	0.657	0.606–0.795	0.0893	0.00500 ± 0.00248	35.3	0.085
28 DAS	0.606	0.426–0.974	0.1117	0.01471 ± 0.00236	28.4	0.331
40 DAS	0.420	0.204–0.842	0.1312	0.02724 ± 0.00363	44.1	0.442

capacity. Six plants pot^{-1} were retained after thinning at 15 days after sowing (DAS). The plants removed while thinning formed the first sample. Subsequently, two plants per pot were sampled at 21, 28 and 40 DAS. Plants in each sample were separated into root (extractable) and shoot, oven dried at 60°C for 3 days and the dry mass then recorded. The roots were fully extracted from the soil at 40 DAS, by washing the soil from the roots. The shoot biomass for each sample was analyzed using the statistical procedure of residual maximum likelihood (ReML) by treating the replications and replications × block effects as fixed and the accessions as random effects to obtain the unbiased estimates of the variance components and the best linear predictions (BLUPs) of the performance of the 252 germplasm accessions and varieties. Heritability in broad sense was estimated as $h^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_e)$. The significance of genetic variability among the accessions was assessed from the standard error of the estimate of genetic variance σ^2_g, assuming the ratio σ^2_g/SE (σ^2_g) to follow normal distribution asymptotically. The salinity susceptibility index (SSI) was calculated following Fisher and Maurer (1978) based on the shoot biomass of each accession.

The SSI and the individual accession means of shoot biomass under salinity stress at 40 DAS were used for clustering the accessions into different classes using Numerical Taxonomy and Multivariate Analysis System (NTSYSPC), version 2.1 (Exeter Software, New York, USA). Similarity/dissimilarity matrix was obtained based on Euclidean distances and thus the accessions were grouped on the basis of UPGMA (unweighted pair-group method of arithmetic average).

Under salinity stress, there was a delay in seedling emergence by 1 or 2 days in all accessions. The reduction in number of seedlings emerged due to salinity stress was marginal with no accession × salinity interaction. The shoot biomass under salinity and the ratio of shoot biomass production under salinity to that of the control showed significant variation at all stages of sampling (Table 1). There was a considerable accession × salinity interaction ($P = 0.001$ for samples at 15, 28 and 40 DAS and $P = 0.05$ for 21 DAS) at all the sampling periods. As wider range of genetic variability for salinity response occurred at 40 DAS, the chickpea accessions were clustered using both SSI and shoot biomass under salinity recorded at 40 DAS. Both the actual productivity under salinity and the SSI are considered equally important. SSI was used to account for the variation of the entries in early growth vigor. The cluster analysis showed four major groups at a similarity coefficient of 75%. The broad sense heritability of shoot biomass production under salinity was considerably high at all stages of sampling (0.65 to 0.79) whereas the ratio of shoot biomass produced under salinity to that of control was relatively low (0.09 to 0.44). The heritability of the latter trait reflects more of the salinity response potential because the growth rates of the accessions are expected to vary depending upon the intrinsic growth vigor and the timing of the exponential growth, and the productivity under salinity is expressed as a fraction of an accession's performance under non-saline conditions. Azhar and McNeilly (1988) reported that the narrow sense heritability value (0.51) estimated for relative root length in sorghum (*Sorghum bicolor*) at 100 mM concentration has been shown to reduce further at 150 mM concentration (0.19). In a relatively more salinity sensitive species such as rice (*Oryza sativa*), the narrow sense (0.198) and broad sense (0.367) heritability values for K/Na ratio, at 12 dS m^{-1} culture medium conditions, were shown to be very low (Gregorio and Senadhira 1993) and close to those measured in our study.

SSI of the accessions was more closely correlated with the shoot biomass under salinity (−0.941) than that of the control (−0.375). The accessions that possessed low SSI and high shoot biomass under salinity stress at 40 DAS were grouped into highly tolerant category and the ones with high SSI and low shoot biomass as highly sensitive (Table 2). The list of accessions under the 'highly tolerant',

Table 2. Cluster group means of salinity susceptibility index (SSI) and shoot biomass under saline condition (100 mM NaCl) at 40 days after sowing and the comparative reaction of 252 chickpea germplasm accessions at ICRISAT, Patancheru, India.

Chickpea accessions	Reaction	SSI	Shoot biomass in control (g plant^{-1})	Shoot biomass in saline treatment (g plant^{-1})
10	Highly tolerant	0.318	0.930	0.756
33	Tolerant	0.606	0.847	0.546
113	Sensitive	0.945	0.729	0.326
96	Highly sensitive	1.318	0.707	0.161

Table 3. Chickpea accessions/genotypes grouped on the basis of salinity susceptibility index (SSI) and shoot biomass production under 100 mM saline water applied condition at 40 days after sowing at ICRISAT, Patancheru, India.

Cluster group	Accession/genotype[1]
Highly tolerant	ICC 10755 (2), ICC 13124 (7), ICC 13357 (8), ICC 15406 (10), ICC 15697 (6), ICCV 92318 (9), ICCV 92337 (5), ICCV 95332 (4), ICCV 95334 (1) and *Jumbo* 2[2] (3)
Tolerant	ICC 1915 (38), ICC 2277 (24), ICC 2919 (29), ICC 4958 (35), ICC 7255 (30), ICC 7272 (12), ICC 7554 (37), ICC 7668 (21), ICC 8151 (47), ICC 8261 (13), ICC 8522 (36), ICC 8855 (23), ICC 9137 (16), ICC 9862 (15), ICCV 10341 (33), ICC 10885 (44), ICC 11879 (25), ICC 12328 (32), ICCV 13523 (27), ICC 13816 (28), ICC 14199 (39), ICC 14595 (17), ICCV 15333 (20), ICC 15510 (19), ICC 15518 (43), ICC 15802 (18), ICC 16796 (34), ICCV 2 (52), ICCV 88202 (26), ICCV 92504 (14), ICCV 95311 (31), ICCV 95333 (11) and ICCV 96329 (22)
Highly sensitive	ICC 283 (171), ICC 440 (153), ICC 637 (228), ICC 708 (203), ICC 762 (192), ICC 1052 (241), ICC 1098 (201), ICC 1161 (194), ICC 1164 (176), ICC 1180 (174), ICC 1397 (163), ICC 1510 (158), ICC 1710 (212), ICC 1715 (200), ICC 1923 (175), ICC 2065 (222), ICC 2072 (180), ICC 2507 (247), ICC 2720 (234), ICC 2884 (250), ICC 2969 (177), ICC 3218 (198), ICC 3230 (162), ICC 3362 (246), ICC 3512 (217), ICC 3631 (245), ICC 3761 (238), ICC 3776 (248), ICC 3946 (249), ICC 4182 (230), ICC 4418 (184), ICC 4463 (240), ICC 4593 (211), ICC 4639 (181), ICC 4657 (179), ICC 4814 (242), ICC 5383 (167), ICC 5434 (220), ICC 5845 (224), ICC 5878 (232), ICC 5879 (237), ICC 6279 (210), ICC 6293 (226), ICC 6537 (168), ICC 6571 (202), ICC 6802 (231), ICC 6816 (214), ICC 7184 (252), ICC 7323 (243), ICC 8058 (197), ICC 8195 (193), ICC 8607 (218), ICC 8621 (166), ICC 9643 (236), ICC 9755 (170), ICC 9848 (207), ICC 10945 (190), ICC 11198 (233), ICC 11584 (187), ICC 11627 (223), ICC 11664 (209), ICC 11944 (244), ICC 12299 (229), ICC 12307 (159), ICC 12537 (199), ICC 12654 (216), ICC 12726 (219), ICC 12824 (213), ICC 12851 (215), ICC 12866 (173), ICC 12916 (239), ICC 12928 (205), ICC 13187 (235), ICC 13283 (208), ICC 13441 (225), ICC 13524 (206), ICC 13628 (188), ICC 13764 (183), ICC 13892 (154), ICC 14077 (195), ICC 14778 (191), ICC 14815 (185), ICC 14831 (165), ICC 15567 (251), ICC 15612 (178), ICC 16269 (189), ICCC 37 (196), ICCL 87322 (204), ICCV 1 (160), ICCV 96752 (164), Chafa (227), E 100YM (221), *Gulabi*[2] (186), JG 62 (172), Myles (169) and Pant G114 (182)

1. Values in parentheses following each accession are the SSI rank out of 252. Accessions showing sensitive reaction are not listed.
2. These were collections from farmers' fields and names are popular among farmers. No accession numbers are available for these entries.

'tolerant' and 'highly sensitive' categories is presented in Table 3. The accessions that were grouped under the highly sensitive category were those that died or were close to mortality under salinity at 40 DAS. The highly tolerant accessions showed less symptoms of salinity effect such as yellowing of the basal leaves in kabuli types or the characteristic anthocyanin pigment appearance in desi types. Most of the highly salinity tolerant entries such as ICCVs 95334, 95332, 92337 and 92318 were kabuli types that were bred at ICRISAT, Patancheru. Majority of the highly sensitive accessions were of desi type. Such screenings were carried out and grouping on the basis of responses were made at the seedling stages in chickpea (Al-Muttawa 2003).

This screening is being planned for repetition during the postrainy season of 2004/05 to confirm the performance of the accessions. Also, determination of various ionic compositions of the plant tissues is being carried out to investigate mechanisms of salt tolerance.

Acknowledgments. The authors gratefully acknowledge the guidance on statistics provided by Subhash Chandra, Senior Scientist (Biometrics and Bioinformatics) and the staff of Genebank and chickpea breeding, ICRISAT for supplying the seeds of mini-core chickpea germplasm and other varieties included in this screening.

References

Al-Muttawa MM. 2003. Effect of salinity on germination and seedling growth of chickpea (*Cicer arietinum* L.) genotypes. International Journal of Agriculture and Biology 5:226–229.

Azhar FM and **McNeilly T.** 1988. The genetic basis of variation for salt tolerance in *Sorghum bicolor* (L) Moench seedlings. Plant Breeding 101:114–121.

Dua RP. 1992. Differential response of chickpea (*Cicer arietinum*) genotypes to salinity. Journal of Agricultural Science 119:367–371.

Dua RP and **Sharma PC.** 1995. Salinity tolerance of kabuli and desi chickpea genotypes. International Chickpea and Pigeonpea Newsletter 2:19–22.

Fisher RA and **Maurer R.** 1978. Drought resistance in spring wheat cultivars. I. Grain yields responses. Australian Journal of Agricultural Research 29:897–912.

Gregorio GB and **Senadhira D.** 1993 Genetic analysis of salinity tolerance in rice (*Oryza sativa* L.). Theoretical and Applied Genetics 86:333–338.

Rengasamy P. 2002. Transient salinity and subsoil constraints to dryland farming in Australian sodic soils: an overview. Australian Journal of Experimental Agriculture 42:351–361.

Upadhyaya HD and **Ortiz R.** 2001. A mini core subset for capturing diversity and promoting utilization of chickpea genetic resources in crop improvement. Theoretical and Applied Genetics 102:1292–1298.

Chickpea Cultivation in Rice-growing Area of Punjab Province of Pakistan: Potential and Constraints

MA Zahid, HR Khan, A Bakhsh and **SM Iqbal** (Pulses Program, National Agricultural Research Centre (NARC), PO NIH, Park Road, Islamabad 45500, Pakistan)

Among various agricultural production systems adopted in Pakistan, rice (*Oryza sativa*)-wheat (*Triticum aestivum*) is extremely important. The total area under rice-wheat is about 1.6 million ha, mostly in the Punjab province. The sustainability of rice-wheat system is under threat in the country due to productivity stagnation, deteriorating soil fertility and increased risk of weeds, pests and diseases (Johansen et al. 2000). The system is inherently exhaustive and disturbs balance of mineral nutrients. Continuous practice of rice-wheat rotation has intensified deficiencies of mineral nutrients (Zia et al. 1992). Development of sustainable cropping systems needs reintroduction of legumes in cereal dominated cropping systems.

Chickpea (*Cicer arietinum*) is the most important food legume grown in Pakistan but its cultivation has traditionally been associated with marginal soils by subsistence farmers under rainfed conditions. The rice-growing belt in Punjab appears to have great potential for chickpea production and its area can be increased through its introduction in the districts Hafizabad, Sheikhupura, Gujranwala, Sialkot and Narowal. But to support chickpea in rice-based system, high-yielding, disease resistant varieties and better management practices for preparation of compacted rice soils are needed (Haqqani et al. 2000). In view of the beneficial role of legumes to enhance sustainability of rice-based system, an attempt was made to generate information on intervention of chickpea in rice-based system and to suggest future research and development needs.

A two-member team of pulses agronomists from the National Agricultural Research Centre (NARC), Islamabad, Pakistan with financial help of the rice-wheat project conducted an informal exploratory survey from 23 February to 1 March 2003 of five major rice-growing districts of Punjab. Overall about fifty experienced farmers and personnel of the Departments of Agriculture Extension and Adaptive Research in these districts were interviewed about the present situation and further prospects of chickpea crop in rice-wheat rotation. The main objectives were to:

- Determine present status of chickpea in rice-growing area and existing chickpea-based cropping systems; and
- Explore possibilities for the reintroduction of chickpea cultivation in rice-wheat cropping system.

Findings

According to the views of agriculture experts and farmers, there is very little scope of pulses in irrigated agriculture in general and that of chickpea in particular. Farmers grow chickpea on limited scale only in drought years as a temporary intervention (Tables 1 and 2). Few farmers grow chickpea and sell the green pods and earn a sizeable income. Farmers adopt rotations involving pea (*Pisum sativum*), potato (*Solanum tuberosum*), onion (*Allium cepa*), fodder and off-season cucumber (*Cucumis sativus*). Rice, wheat, sugarcane (*Saccharum officinarum*) and sunflower (*Helianthus annuus*) are the main mandate crops in the area and every training program of farmers at village level is designed according to the needs of these crops. Introduction of chickpea in the area requires a