Tapping the large genetic variability for salinity tolerance in chickpea

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Abstract

Salinity is an ever-increasing problem in agriculture worldwide and especially in Australia. Improved genotypes that are well adapted to saline conditions are needed to enhance and sustain production in these areas. A screening of 263 accessions of chickpea, including 211 accessions from ICRISAT's mini-core collection (10% of the core collection and 1% of the entire collection), showed a six-fold range of variation for seed yield under salinity, with several genotypes yielding 20% more than the previously-released salinity tolerant cultivar CSG8962. No significant relation was found between biomass at the late vegetative stage and final seed yield under salinity. Performance of seed yield under salinity was explained in part by the yield potential under control conditions, and a salinity tolerance component. The major trait related to salinity tolerance was the ability to maintain under salinity a large number of viable pods with seeds. In contrast, the relative seed size under salinity did not differ between tolerant and sensitive genotypes. Preliminary analysis of genotypic data for approximately 50 SSR markers on 211 genotypes revealed some associations with salinity tolerance that deserve a detailed analysis. Future effort should focus on the effect of salinity on the reproductive stage of development.

Key Words

Chickpea, salinity tolerance, seed yield, large screening, mini-core collection

Introduction

Salinity affects 100 million hectares of arable lands worldwide and this area is expanding (Ghassemi et al., 1995). In Australia and India, salinity has become a major deterrent to crop production including legumes. Therefore, breeding salinity tolerant cultivars would greatly help farming in chickpea growing area affected by salinity.

Chickpea is very sensitive to salinity (Lauter and Munns, 1986). Previous results by Dua (1992) showed that no chickpea could grow at EC levels higher than 6 dS, although this work refers to soils that were also high in pH (8.8). To improve the adaptation of chickpeas to saline soils, it is critical to identify tolerant sources and understand the genetic basis of salinity tolerance. It has been previously stated that there was too little variability in chickpea for salinity tolerance to allow successful breeding of salinity tolerant varieties (Saxena, 1984, Johansen et al., 1990). However, only limited genotypes were used to test that hypothesis, and it is very likely that more variation may be evident by testing a wider range. Indeed, Maliro and colleagues (2004) have found variation for salinity tolerance at early vegetative stage.

Therefore, the objectives of this study were to identify salinity tolerant genotypes from the chickpea mini-core collection at ICRISAT, representative of the chickpea germplasm, and genotypes reported to perform well under salinity, to explore potential tolerance mechanisms in chickpea, and identify SSR alleles linked to salinity tolerance using association mapping based on phenotypic and genotypic data from 35 SSR markers.

Methods

Protocol. Plants were grown under saline and non-saline conditions in 27 cm diameter pots containing 7.5 kg of Vertisol soil. The experiment was carried out between November 2004 and March 2005 at ICRISAT headquarters (Patancheru, AP, India) in an open-air facility equipped with a rainout shelter facility. The saline treatment was applied as an 80 mM solution of NaCl in a sufficient volume to saturate the soil at field capacity. This corresponded to an addition of 1.875 L of a 80 mM NaCl to each pot. The saline treatment was applied at sowing. Thereafter, pots were watered with tap water containing no significant amount of salt, and maintained close to field capacity (gravimetrically) to avoid an increase in salt concentration. The bottom of the pots was sealed to avoid any salt leakage. Non-saline treated controls were watered with non-saline water. In both treatments, six seeds were planted in each pot and later thinned to 4 plants per pots. Two experiments were planted side by side: one for the evaluation of biomass at 50 DAS, one for the evaluation of yield. In each exp, the design was a RBD with 2 factors (salt and control). In each experiment, 3 replicated pots per treatment (salinity and control) and genotype were used. A total of 263 genotypes were tested, which included 211 accessions from the mini-core collection of ICRISAT (10% core collection, 1% entire collection (Upadhyaya and Ortiz, 2001)), chickpea lines reported as tolerant to sodicity (Dua and Sharma, 1995), popular varieties and breeding lines, and one cultivar previously released by the Indian Institute of Pulse Research (IIPR) for salinity tolerance (CSG8962). Both kabuli and desi types were included in the study.

Measurements. Parameters measured included: time to flowering and maturity (d), shoot biomass at 50 DAS (in g plant^{-1}), seed yield at maturity (g pot^{-1}), 100-seed weight, and pod number per plant. To assess which trait was potentially related to salinity tolerance (see below), the relative reduction in shoot biomass at 50 DAS, seed number per plant, and 100-seed weight were calculated as: value under salinity/value of control.

Predicted yield (Ŷs). A highly significant linear relationship was found between seed yield under salinity (Ys) and seed yield under control (Yc) (r^2 = 0.50, data not shown). Therefore, the seed yield performance under salinity couldn't be attributed to the salinity tolerance of genotypes alone, but to a yield potential (Yc) component plus a residual. The residual would then account for salinity tolerance *per se* plus error, and represent the part of variation in yield under salinity that is not explained by yield potential, using a similar approach to Bidinger et al (1987). Predicted yield under salinity (Ŷs) was calculated based on the relation Ys = aYc + b, where a and b were found to be 0.45 and 2.07, respectively. Residuals (R) were computed as the difference between Ys and Ŷs (Ys – Ŷs), and used as a proxy for salinity tolerance *per se.*

Results and discussion

Seed yield performance under salinity. There was a large variation, close to 6-fold range, in the seed yield under saline conditions across genotypes, with lowest yield being as low as 2 g pot^{-1}, whereas the highest yield was 12 g pot^{-1}. Three genotypes had 20% higher yield (and residuals between 2.8 and 3.5) than the previously identified salt tolerant genotype CSG8962 (residual = 1.8) (Fig. 1). These results showing a large genotypic variability are in contrast with previous findings that there is no variation for salinity tolerance in chickpea. Interestingly, we found a large contrast in seed yield under salinity between the parents of an existing RIL population. Genotype ICCV2, an extra-short duration genotype showed poor performance under salinity (yield = 4.5 g pot^{-1} and residual = -4.8), whereas JG62 reached 10.8 g pot^{-1} (residual = 0.4), about 2.4 fold that of ICCV2. Good sources of variation in seed yield under salinity were found both in desi and kabuli genotypes.

We found no relation between seed yield under salinity and the biomass at 50 DAS (data not shown). Figure 2 illustrates the type of response of chickpea to salinity stress with tolerant and sensitive genotypes that may show a very similar biomass, although the number of pods set is much higher in the tolerant material. This result indicates that the reproductive structures are suffering more salinity than biomass production and that further progress in salinity tolerance research in chickpea may come from understanding the effect of salinity on reproduction and pod set, but not from the evaluation at the vegetative stage as has been previously done (Maliro et al., 2004).

We also carried out a preliminary analysis in which, first, simple linear regressions were run to identify promising markers. These were then used in a model selection based on step-wise regression. Several SSR markers were found to have a significant association with both seed yield under control and seed yield under salinity, whereas others markers were found to be associated only with seed yield under salinity. A more detailed and systematic analysis of putative marker-trait association is under way.

Potential parameters explaining salinity tolerance. We used the standardized residual (Ŷs – Ys) to explore potential mechanisms of tolerance. Above we found that reproductive structures were likely to be more affected by salinity than biomass production. In agreement with that, we found no correlation between the relative reduction in shoot biomass at 50 DAS and the residual (data not shown). We then tested whether seed setting or seed development was more affected by salinity, by correlating the relative decrease in these two parameters under salinity to the standard residual computed above. We found that the residuals were very closely related to a relative decrease in seed number (r^2 = 0.65, Figure 3), but they were not related to a decrease in seed size (data not shown). These results indicate that the sensitivity of chickpea to salinity may be limited to a very short period around reproductive phase. However, we do not have data to establish whether seed or pod set is more affected by salinity. More work is needed to determine which key organ or reproductive process is primarily affected.

We computed the mean residual for desi and kabuli genotypes and found a higher value for desi (0.14) than for kabuli types (-0.48), showing that desi genotypes may have a better tolerance to salinity, in agreement with previous findings (Dua and Sharma, 1995).

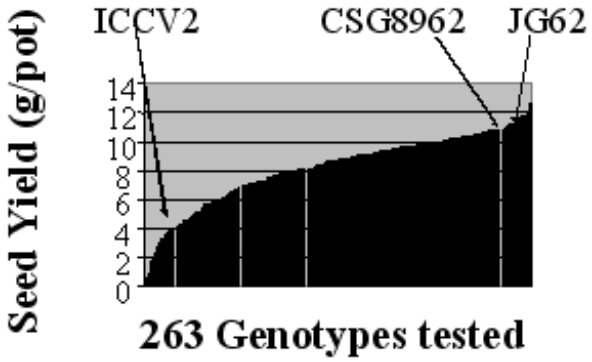

Figure 1. Seed yield (g pot-1) of 263 chickpea genotypes, including the mini-core collection (1% full collection, 10% core collection) of ICRISAT.

Figure 2. Aerial biomass and the pod setting under salinity of a salinity tolerant and salinity sensitive genotype. The photograph shows that biomass can be very similar even though pod set can be differentially affected by salinity.

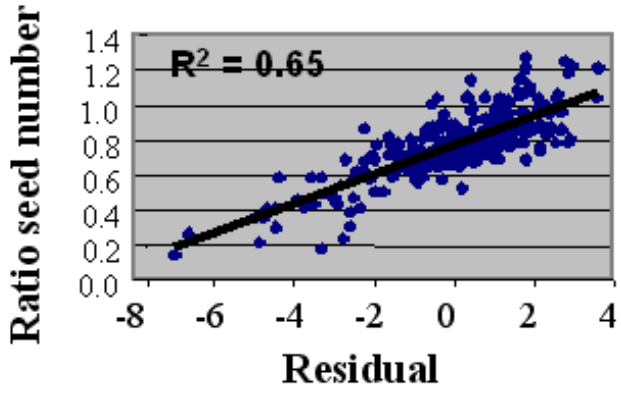

Figure 3. Relation between the residual (difference between observed and predicted yield) under salinity (Y-Ŷs)), and the ratio of seed number.

Conclusion

This study revealed the availability of a large variation in seed yield under salinity in chickpea germplasm, although previous research stated the contrary (Saxena, 1984). These variations could only be truly assessed by measuring seed yield under salinity, as vegetative biomass had strictly no relation with seed yield, and suggests that reproductive structures are likely to be more affected by salinity than biomass production. We also found that about half of the variation in seed yield under salinity can be explained by yield potential. Therefore, we found that the salinity tolerance *per se*, which we proxied as the difference between estimated and observed seed yield under salinity, was more related to the ability to maintain a large seed number than differences in seed size.

The variation in salinity tolerance identified is sufficiently large to open the possibility of breeding for salinity tolerance in chickpea. Further research is needed to phenotype and genotype the RILs (ICCV2 x JG62) under control and saline conditions, together with an attempt to identify QTLs for salinity tolerance in chickpea, with the aim of using marker assisted selection. Preliminary data from association mapping revealed some association between marker data and seed yield under salinity and/or seed yield under control conditions.

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