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Large variation in salinity tolerance in chickpea is explained by differences in sensitivity at the reproductive stage

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Abstract

Salinity is an ever-increasing problem in agriculture worldwide, especially in South Asia (India, Pakistan) and 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 6-fold range of variation for seed yield under salinity (1.9 L of 80 mM NaCl per 7.5 kg Vertisol), with several genotypes yielding 20% more than a previously released salinity tolerant cultivar. The range of variation in yields under salinity was similar in both kabuli and desi chickpeas, indicating that breeding for salinity tolerance can be undertaken in both groups. A strong relationship ($r^2 = 0.50$) was found between the seed yield under salinity and the seed yield under a non-saline control treatment, indicating that the seed yield under salinity was explained in part by a yield potential component and in part by salinity tolerance *per se*. Seed yields under salinity were therefore computed to separate the yield potential component from the residuals that accounted for salinity tolerance *per se*. Among the genotypes evaluated, desi genotypes had higher salinity tolerance than kabuli genotypes. The residuals were highly correlated to the ratio of seed yield under salinity to that of the control, indicating that both parameters can be used to assess salinity tolerance. A similar ratio was calculated for shoot dry weight at 50 days after sowing. However, no significant correlation was found between the shoot dry weight ratio and the yield ratio, indicating that differences in salinity tolerance among genotypes could not be inferred from measurements in the vegetative stage. The major trait related to salinity tolerance was the ability to maintain a large number of filled pods, whereas seed size was similar in tolerant and sensitive genotypes. Salinity tolerance was not related to the shoot Na^+ or K^+ concentrations.

Keywords: Chickpea; *Cicer arietinum*; Salinity tolerance; Seed yield; Large screening; Mini-core collection

1. Introduction

Salinity affects about 100 million ha of arable lands worldwide and this area is expanding dramatically (Ghassemi et al., 1995). In Australia and India, salinity has already become a major deterrent to crop production, including legumes. In Australia, salinity is likely to affect 17 million ha by 2050 according to a recent report (ANRA, 2001). In India alone, about 13 million ha are currently affected by salinity (Consortium for Unfavorable Rice Environment, IRRI, 2003). Although agricultural management options are available and policies could be implemented, for example, in relation to the use of irrigation water, such options often contrast with the immediate economic choices of farmers. Therefore, a more practical and immediate option is the breeding of salinity tolerant cultivars.

Chickpea (*Cicer arietinum* L.) is very sensitive to salinity (Lauter and Munns, 1986). Previous results by Dua (1992) showed that no chickpea variety could grow at EC levels higher than 6 dS/m, although this work was done in 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 is too little variability for salinity tolerance in chickpea to undertake a successful breeding program for salinity tolerance (Saxena, 1984; Johansen et al.,

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1990). However, only a limited number of genotypes were used to test this hypothesis, and it is very likely that more variation may be evident by testing a wider range of germplasm. Indeed, Maliro et al. (2004) found large variation for salinity tolerance at the early vegetative stage within 200 accessions of chickpea, including 19 wild relatives. Large variations have also been found for vegetative biomass across 252 chickpea accessions (Serraj et al., 2004). One drawback in these previous studies was that genotypes were assessed for salinity tolerance based on vegetative biomass and not on seed yield. Knowing the high sensitivity of reproductive stages to abiotic stresses (Boyer and Westgate, 2004; Leport et al., 2006), we raise the question here whether the success of a genotype under salinity is bound to the reproductive success under that stress.

Therefore, the objectives of this study were to identify salinity tolerant chickpea genotypes based on their seed yield under salinity, and to explore potential tolerance mechanisms. To make sure we encompassed a large pool of genetic diversity, we evaluated: (i) the chickpea mini-core collection developed by ICRISAT that contains ~10% of the core collection or ~1% of the entire collection (Upadhyaya and Ortiz, 2001), representative of the chickpea germplasm; (ii) genotypes previously reported to perform well under sodic soils (Dua and Sharma, 1995); and (iii) elite breeding lines.

2. Materials and methods

2.1. Plant growth and treatment conditions

Plants were grown under saline and non-saline conditions in 0.27 m diameter pots containing 7.5 kg of Vertisol soil taken from the ICRISAT farm [pH 8.1, CEC/clay ratio = 0.87, electric conductivity = 0.10 mmhos cm^{-1} (El Swaify et al., 1985)], fertilized with di-ammonium phosphate (DAP) at a rate of 300 mg kg^{-1} soil. The experiments were carried out between November 2004 and March 2005 at ICRISAT headquarters (Patancheru, AP, India) in an open-air facility equipped with a rainout shelter. The average maximum temperatures ranged between 29.7 and 32.6 °C and minimum temperatures ranged between 15.4 and 16.1 °C. The saline treatment was applied as an 80 mM solution of NaCl in a sufficient volume to wet the soil to field capacity. This corresponded to an addition of 1.875 L of an 80 mM NaCl solution to each pot, i.e. an application of 8.77 g NaCl pot^{-1}, equivalent to 1.17 g NaCl kg^{-1} soil. The saline treatment was applied at sowing. Thereafter, pots were watered with tap water containing no significant amount of NaCl, and maintained close to field capacity (determined gravimetrically) to avoid an increase in salt concentration in the soil solution. The bottom of the pots was sealed to avoid any salt leakage. Non-saline treated controls were brought initially to field capacity with non-saline water.

In both treatments, six seeds were planted in each pot and later thinned to four plants per pot. Two experiments were planted side by side: one for the evaluation of biomass at 50 days after sowing (DAS), one for the evaluation of seed yield. In each experiment, the design was a randomised block design with two factors (salt and control) and three replicates. A total of 263 genotypes were tested, which included 211 accessions from the mini-core collection of ICRISAT [10% of the core collection, 1% of the entire collection (Upadhyaya and Ortiz, 2001)], chickpea lines reported as tolerant to sodicity (Dua and Sharma, 1995), popular cultivars and breeding lines, and one cultivar previously released by the Central Soil Salinity Research Institute (CSSRI) for salinity tolerance (CSG8962). Both kabuli ($n = 58$) and desi types ($n = 192$) were included in the study (the remaining 13 genotypes were intermediates between desi and kabuli types).

2.2. Measurements

Parameters measured included: time to flowering and maturity (days, taken on a pot basis, recorded when two or more plants per pot had reached flowering/maturity), shoot biomass at 50 DAS (g pot^{-1}), seed yield at maturity (g pot^{-1}), 100-seed weight, and pod number per plant. We also measured the Na^+ and K^+ concentrations in the shoot from the vegetative biomass evaluation experiment. For this, 150 mg of finely ground shoot samples (leaf, stem, plus flowers if present) were digested in 4 ml of concentrated sulphuric acid with 0.5% selenium powder at 360 °C for 4500 s on a block digester and the digest was diluted to 75 ml using distilled water. This dilution was used to estimate Na^+ and K^+ (Sahrawat et al., 2002) using an atomic absorption spectrophotometer (Varion model 1200, Australia).

2.3. Predicted yield ($\hat{Y}_s$) and salinity tolerance indexes

A highly significant linear relationship was found between seed yield under salinity (Y_s) and seed yield under control (Y_c) ($r^2 = 0.50$, Fig. 1). Therefore, the seed yield performance under salinity could not be attributed to the salinity tolerance of genotypes alone, but to a yield potential component plus a residual. That 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). To compute these residuals, i.e. salinity tolerance *per se*, the predicted yield under

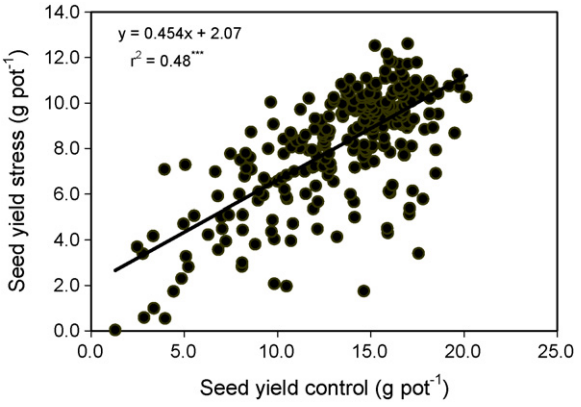

Fig. 1. Relationship between seed yield in the non-saline control and seed yield under salinity [g pot^{-1} (four plants in both treatments)]. The regression equation was used to compute the estimated yield ($\hat{Y}_s$).

salinity ($\hat{Y}_s$) was calculated based on the relation between Y_c and Y_s, such as:

$$Y_s = aY_c + b \quad (1)$$

where a and b were found to be 0.45 and 2.07, respectively (Fig. 1) ($r^2 = 0.48$). Residuals (R) were computed as the difference between Y_s and $\hat{Y}_s$ (Y_s–$\hat{Y}_s$), and used as a proxy for salinity tolerance *per se*.

After computing these residuals, they were regressed as dependant variables, against a number of explanatory variables, to identify traits potentially related to salinity tolerance, or traits easier to assess. Variables included: (i) the ratio of seed yields (Y_s/Y_c); (ii) the ratio of shoot biomass at 50 DAS (shoot biomass under salinity/shoot biomass under non-saline conditions); (iii) the ratio of seed number per plant (seed number under salinity seed number under non-saline); (iv) the ratio of 100-seed weight (100-seed weight under salinity/100-seed weight under non-saline); (v) Na^+ and K^+ concentration in the shoot; and (vi) time to flowering, using explanatory variable in a Type 2 polynomial equation.

3. Results

3.1. Seed yield and biomass accumulation under salinity

A highly significant linear relationship was found between seed yield under salinity (Y_s) and seed yield under non-saline conditions (Y_c) ($r^2 = 0.50$, Fig. 1). We also found a very close relation between the residuals (Y_s–$\hat{Y}_s$) and the ratio of seed yield (Y_s/Y_c) ($r^2 = 0.81$, Fig. 2). Therefore, residuals and ratio of seed yield (Y_s/Y_c) were both used as proxies for salinity tolerance in the remaining analyses.

There was a large variation, close to a 6-fold range, across genotypes in the seed yield under saline conditions, with lowest yield being 2 g pot^{-1} and the highest yield 12 g pot^{-1} (Fig. 3, Table 1). Three genotypes had about 20% higher yield than the previously identified salt tolerant genotype CSG8962 (Table 1). The residuals for each genotype also showed a large range of variation for salinity tolerance, i.e. from −7.0 to 3.6. The three genotypes that yielded the most under salinity, ICC 5003, ICC15610, and ICC1431, had residuals ranging between 2.8

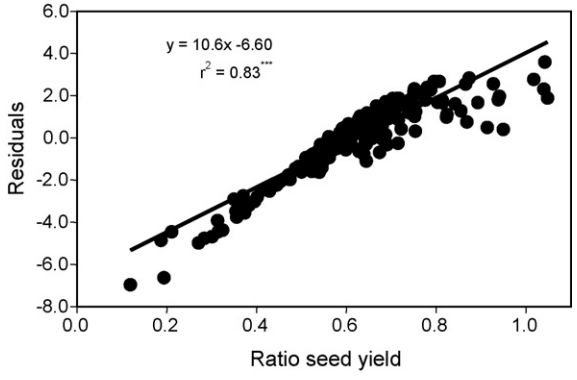

Fig. 2. Relationship between the ratio of seed yield (Y_s/Y_c) and the residuals [difference between observed and predicted yield under salinity (Y_s–$\hat{Y}_s$)], which were computed from Fig. 1.

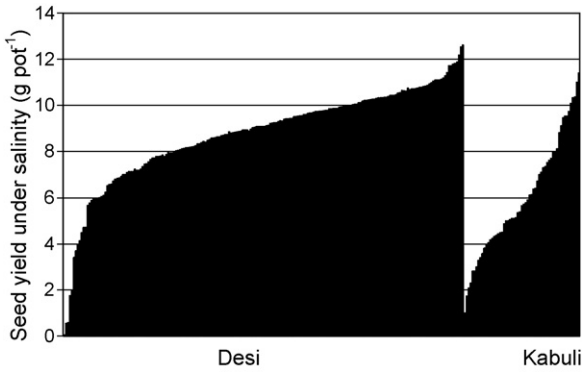

Fig. 3. Seed yield (g/pot) of 192 desi and 58 kabuli chickpea genotypes, including 211 genotypes from the mini-core collection of ICRISAT, under salinity conditions.

and 3.5 indicating that their yield was largely due to a high salinity tolerance, whereas CSG8962 had a residual of 1.8, showing a slightly lower degree of tolerance than the top three genotypes. The mean residual of all desi genotypes was 0.30, which was higher that the mean residual of all kabuli genotypes (−0.90), indicating that the desi genotypes had more salinity tolerance than the kabuli genotypes assessed. This was also seen by the predominance of desi among the top most tolerant lines and the higher representation of kabuli in the top most sensitive lines (Table 1). Yet, good sources of variation in seed yield under salinity were found in both desi and kabuli chickpeas, with each type showing about a 6-fold range in seed yield in the genotypes selected (Fig. 3, Table 1).

Interestingly, we found a large contrast in seed yield under salinity between the parents of an existing RIL population developed from a cross ICCV2 and JG62 to determine map positions of genes conferring double podding and seed traits in chickpea (Muehlbauer, 2002; Cho et al., 2002), making it possible to use this population to search for QTLs for salinity tolerance (Table 1). The genotype ICCV2, an extra-short duration genotype showed poor performance under salinity (seed yield = 4.5 g pot^{-1}), while JG62 had a seed yield of 10.8 g pot^{-1}, about 2.4-fold that of ICCV2. The residuals of the parents of the mapping population also contrasted for salinity tolerance, with salinity sensitive ICCV2 having a residual of −4.8, whereas the tolerant genotype JG62 had a residual of 0.40.

The ratio of seed yield under salinity (Y_s/Y_c) and the ratio of shoot dry weight at 50 DAS (shoot dry weight under salinity/shoot dry weight under non-saline) were 0.61 and 0.65, respectively, across the 263 genotypes tested, showing that both biomass at 50 DAS and seed yield at maturity were similarly reduced by salinity. Therefore, we determined whether salinity tolerance, assessed by the yield ratio Y_s/Y_c was correlated with measurements at the vegetative stage by comparing Y_s/Y_c to the ratio of shoot dry weight at 50 DAS. We found no correlation between the ratio of seed yield and the ratio of shoot dry weight at 50 DAS (Fig. 4). Indeed, some genotypes with similar biomass under saline conditions had very different pod numbers. In fact, it was clearly visible in the screening experiments, and from Table 1, that genotypic differences in

Table 1
List of the 15 most tolerant and 10 most sensitive accessions in the screening for salinity tolerance, along with similar information for CSG8962, JG62, ICCV2, giving type, number of days to flowering under salinity, total dry mass (TDM) under control and salinity and seed yield under control and salinity

Genotype	Type	Day to flower (DAS)	TDM (g pot^{-1})		Seed yield (g pot^{-1})	
			Control	Salinity	Control	Salinity
Tolerant						
ICC1431	Desi	69	35.32 ± 9.36	29.29 ± 3.87	16.99 ± 3.01	12.62 ± 1.51
ICC15610	Desi	75	43.60 ± 2.19	28.63 ± 5.04	15.23 ± 2.60	12.53 ± 2.00
ICC5003	nd	63	34.46 ± 8.44	23.81 ± 2.18	15.87 ± 2.92	12.17 ± 0.66
ICC4593	Desi	61	37.50 ± 2.89	26.86 ± 5.28	15.99 ± 1.11	11.87 ± 0.83
ICC12155	Desi	66	35.23 ± 0.71	26.11 ± 4.25	16.15 ± 2.60	11.82 ± 1.08
ICC2580	Desi	57	36.74 ± 3.61	23.68 ± 1.67	17.47 ± 0.61	11.79 ± 0.80
ICC67	Desi	58	39.34 ± 5.09	24.01 ± 1.81	16.98 ± 2.43	11.72 ± 0.61
ICC11121	Desi	64	35.06 ± 4.59	24.51 ± 1.91	16.52 ± 0.51	11.71 ± 1.06
ICC8950	Desi	59	35.97 ± 2.73	23.35 ± 3.99	15.77 ± 0.83	11.41 ± 1.32
L 550	Kab	61	36.57 ± 3.20	25.34 ± 4.06	16.63 ± 2.17	11.40 ± 1.83
ICCV10	Desi	60	35.35 ± 3.20	24.26 ± 3.00	19.68 ± 0.44	11.27 ± 4.12
ICC9942	Desi	63	34.38 ± 10.37	23.54 ± 3.22	16.76 ± 2.34	11.18 ± 1.39
ICC867	Desi	57	33.66 ± 2.40	24.33 ± 2.88	16.20 ± 0.60	11.13 ± 1.42
JG11	Desi	38	34.98 ± 3.98	19.11 ± 7.08	19.78 ± 2.64	11.10 ± 3.33
ICC4495	Desi	66	34.46 ± 3.64	25.21 ± 5.97	14.76 ± 3.63	11.09 ± 2.19
CSG8962	nd	64	38.15 ± 3.84	27.10 ± 2.19	16.52 ± 0.62	10.62 ± 0.61
JG62	Desi	53	32.80 ± 4.42	17.89 ± 2.12	18.30 ± 2.17	10.81 ± 1.32
Sensitive						
ICC6306	Desi	98	42.50 ± 4.89	29.75 ± 2.72	1.29 ± 0.58	0.24 ± 0.26
ICC8522	Desi	82	43.41 ± 4.22	34.17 ± 1.11	3.97 ± 1.22	0.55 ± 0.34
ICC1915	Desi	91	41.09 ± 6.51	32.52 ± 2.49	2.84 ± 1.41	0.58 ± 0.35
ICC13357	Kab	86	39.68 ± 4.47	26.99 ± 4.63	3.36 ± 1.86	1.00 ± 0.81
ICC8058	Kab	77	40.62 ± 10.08	23.31 ± 6.39	9.83 ± 5.33	1.50 ± 1.83
ICC15518	Kab	81	40.14 ± 4.69	28.97 ± 4.44	4.42 ± 1.70	1.73 ± 0.88
ICCV96029	Desi	30	25.44 ± 3.23	3.78 ± 2.09	14.63 ± 1.40	1.75 ± 1.35
ICC3946	Desi	84	38.93 ± 3.37	18.13 ± 7.15	10.48 ± 1.80	1.96 ± 3.17
ICC10885	Kab	81	43.46 ± 4.35	23.27 ± 2.25	4.85 ± 1.23	2.30 ± 2.04
ICC5337	Kab	87	38.96 ± 6.01	28.08 ± 2.59	5.21 ± 1.79	2.81 ± 1.17
ICCV2	Kab	37	27.30 ± 7.06	9.00 ± 0.84	15.88 ± 2.74	4.51 ± 1.28

Data are means ± S.D. of three replicated pots; nd, not determined

vegetative biomass under salinity were small whereas differences in seed yield and in particular number of pods per plants under salinity showed dramatic variations between plants. This was shown by a much better distribution of the ratio of yield under salinity (Y_s/Y_c) across the range of values (0.2–1.0), whereas the ratio of shoot dry weight at 50 DAS ranged between 0.4 and 1.4 but most of the genotypes were between 0.5 and 0.8, showing limited discrimination. Both ratios had similar average values across the 263 genotypes tested.

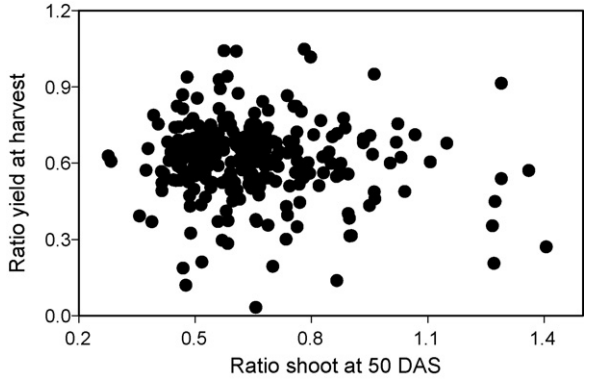

Fig. 4. Relationship between the ratios of shoot dry weight at vegetative stage (shoot dry weight under salinity/shoot dry weight under control) and the ratio of seed yield (Y_s/Y_c).

We also carried out a preliminary map-free trait-marker association analysis in which simple linear regressions were run to identify promising markers. These were then used in a model selection based on step-wise regression. Several single sequence repeat (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 (data not shown). A more detailed and systematic analysis of putative marker-trait association is underway.

3.2. Potential parameters explaining salinity tolerance

The standardized residuals of seed yield (Y_s–$\hat{Y}_s$) were used to explore the potential mechanisms of tolerance. In agreement with the lack of relation between the ratio of seed yield and the ratio of shoot dry weight found above, there was no correlation between the ratios of shoot dry weight at 50 DAS and the residuals of seed yield (data not shown) indicating that differences in salinity tolerance in the vegetative stage did not translate into seed yield at maturity. We also tested whether

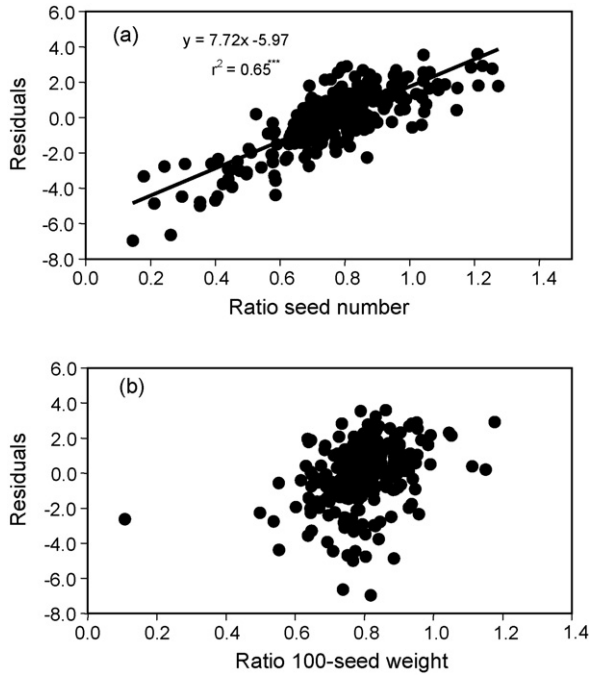

Fig. 5. Relationship between the residuals [difference between observed and predicted yield under salinity (Y–$\hat{Y}_s$)] and: (a) the ratio of seed number, and (b) the ratio of 100-seed weight.

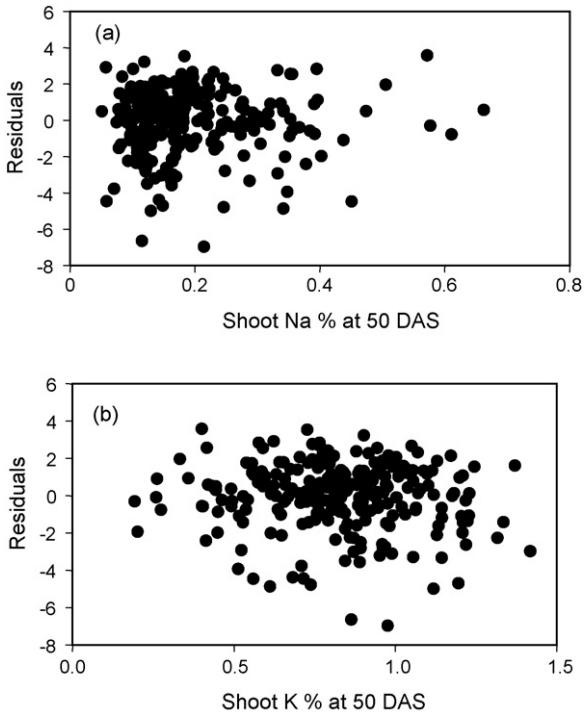

Fig. 6. Relationship between the residuals [difference between observed and predicted yield under salinity (Y–$\hat{Y}_s$)] and: (a) the Na^+ concentration, and (b) K^+ concentration in the shoots.

differences in the residuals among genotypes were due to differences in seed set or seed development. When the relative decrease in seed number and seed size under salinity was regressed against the standard residual computed above, the residuals were very closely correlated with the relative decrease in seed number (seed number under salinity/seed number under control) ($r^2 = 0.65$, Fig. 5a), but they were not correlated with a relative decrease in seed size (100-seed weight under salinity/100-seed weight under control) (Fig. 5b). Since kabuli are bigger seeded than desi, the seed filling of kabuli may be more affected than in desi. Therefore, we tested the same hypothesis as above, by separating kabuli and desi types, and found essentially the same pattern of relation, i.e. a significant relation between the residuals and the ratio of seed number, but no relation between the residuals and the ratio of 100-seed weight.

We also tested whether the differences in salinity tolerance could be explained by differences in accumulation in Na^+ and K^+ at the vegetative stage just prior to flowering (most of the genotypes had lost a large portion of their leaves at maturity). No significant correlation was found between the residuals and either shoot Na^+ (Fig. 6a) or shoot K^+ concentration (Fig. 6b). Similarly, no significant correlation was found between the ratio of seed yield and either shoot Na^+ or shoot K^+ concentration (data not shown). We also found no significant correlation between the ratios of shoot dry weight under salinity at 50 DAS and shoot Na^+ concentration or shoot K^+ concentration (data not shown).

Finally, we looked at a possible relation between salinity tolerance and the maturity type of the considered genotypes. The hypothesis was that late maturing genotypes would be exposed to salinity stress for longer duration, which might make them more susceptible than early maturing types. The relation between the residuals and the number of days to flowering showed a polynomial pattern (Fig. 7). The relationship was statistically significant ($r^2 = 0.24$, $P < 0.01$), but weak. A few extra-early and many late-maturing genotypes were found to be the least tolerant types.

4. Discussion

We found that a large range of variation existed for seed yield under salinity and that this variation was due to the yield potential of the genotypes and their salinity tolerance *per se*. Though the range of seed yield under salinity was similar in desi and kabuli chickpeas, salinity tolerance *per se* was found to be slightly higher in desi than in kabuli types. Salinity tolerance appeared not to be correlated with seed size, but highly

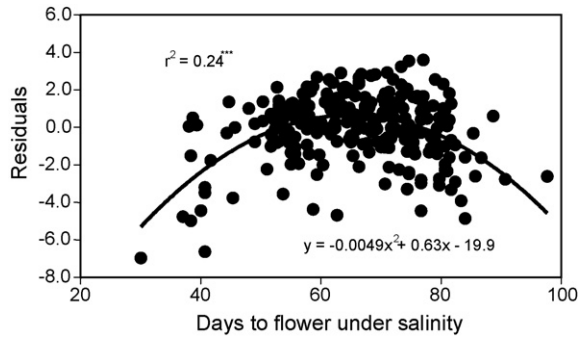

Fig. 7. Relationship between the residuals [difference between observed and predicted yield under salinity (Y–$\hat{Y}_s$)] and flowering time (days after sowing) under salinity.

correlated to the relative decrease in seed number. Salinity tolerance was not related to the ratio of shoot biomass, nor to the Na^+ or K^+ concentration in shoot.

These results showing a large genotypic variability contrast with previous reports (Saxena, 1984; Johansen et al., 1990) that the variation for salinity tolerance was inadequate to achieve worthwhile progress in breeding for salt tolerance. However, the small sample sizes of these earlier studies may explain the limited genotypic range observed. In the present work, we have assessed the mini-core collection of ICRISAT (Upadhyaya and Ortiz, 2001), which represents most of the possible variability in chickpea germplasm, and included genotypes previously reported as tolerant to sodicity (excess Na^+ and high pH). Using that large range of diverse germplasm, we have clearly demonstrated that a large range of variation for salinity tolerance is available for use in breeding programs.

Other previous studies have also demonstrated that genotypic variation exists for salinity tolerance in chickpea (Serraj et al., 2004; Maliro et al., 2004). However, tolerance in these studies was assessed based on biomass production at the vegetative stage, a salinity susceptibility index (SSI) based on vegetative biomass, and leaf scoring. However, no yield assessment under salinity was performed. In this work, the biomass production under saline conditions at the vegetative stage (50 DAS) was about 35% of that under non-saline conditions, whereas the seed yield was about 60% of that in non-saline conditions. The biomass under saline conditions at maturity was about 65% of that in non-saline conditions. These data show that salinity induces a delay in the early plant development, in agreement with previous findings (Serraj et al., 2004). Indeed, flowering time was delayed on average by 8 days under salinity. However, we found no relation between the ratio of biomass production at the vegetative stage and the ratio of seed yield under salinity, which is in agreement with previous findings by Dua and Sharma (1995). These data show that both biomass and yield are equally affected by salinity, but that genotypic differences in salinity tolerance are not explained by genotypic differences in biomass production. Therefore, these results strongly suggest that evaluation for salinity tolerance at the vegetative stage is not a suitable screening tool for yield under salinity in chickpea.

We found that the residuals, which accounted for salinity tolerance *per se*, were closely related to the relative decrease in seed number per plant, but not to the relative decrease in seed size. These results indicate that the sensitivity of chickpea to salinity may be limited to a very short period in the reproductive phase, and that once pods and seeds are set, their development is affected very little by salinity. Indeed the 100-seed weight under salinity across all genotypes was 80% of that in control. We presently do not have data to establish whether flower, seed or pod set was more affected by salinity. More work is needed to determine which key organ or reproductive step is primarily affected.

Desi chickpea types were found relatively more tolerant than kabuli types, since residuals for desi (0.30) were higher than residuals for kabuli (−0.90). This is contrary to what was previously reported in chickpea by Dua and Sharma (1995). However, the work by Dua and Sharma (1995) was carried out under sodic soil conditions (high Na^+ and high pH), whereas the current work was done under excess Na^+ only, which might explain the differences. It is interesting to note that desi chickpea also seem to achieve a higher harvest index under water deficit than kabuli chickpea (Krishnamurthy, pers. comm.). We speculate that there might be some particular steps in the reproductive development that make desi chickpea better suited than kabuli to cope with abiotic stresses such as drought and salinity that share some commonalities (Leport et al., 2006). That particular step could be equally affected by water deficit and salt stress.

It was interesting, but also intriguing, that salinity tolerance was not related to the Na^+ and K^+ concentration in the shoot, though many previous reports on salinity show a good relationship between salinity tolerance and Na^+ accumulation. We did not find any relation either between the relative decrease in shoot biomass at vegetative stage and Na^+ accumulation, which meant that Na^+ accumulation was clearly not the cause for differences in salinity tolerance. In fact, the shoot Na^+ concentration ranged from 0.05 to 0.66%, with only 8 out of 263 genotypes with concentrations above 0.40%. Even a concentration of 0.40% Na^+ would correspond to a molar concentration of 17 mM assuming fresh tissue contains about 10% dry weight. Such a concentration remains relatively modest and is unlikely to cause any major toxic effect on the plants (Fricke, 2004). The fact that previous reports assessed salinity tolerance based on the reduction in shoot biomass under salinity, and then found that there was a good relation with the Na^+ accumulation, may raise some doubt about the real value of using Na^+ concentration as a proxy for salinity tolerance.

Although preliminary data from association mapping revealed some association between marker data and seed yield under salinity and/or seed yield under non-saline conditions, further work is needed to phenotype and genotype the RILs (ICCV2 × JG62) under saline and non-saline conditions, and to identify QTLs for salinity tolerance in chickpea, with the aim of using marker-assisted selection.

5. 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 ratio had strictly no relation with seed yield ratio, and suggests that differences in the sensitivity of the reproductive steps are likely to explain most of the differences found in salinity tolerance. Indeed, 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.

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