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## **Consistent Variation across Soil Types in Salinity Resistance of a Diverse Range of Chickpea (*Cicer arietinum* L.) Genotypes**

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**Keywords** Sodium chloride; seed yield; pod number; seed number; seed size; Alfisol; Vertisol.

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## **Abstract**

Chickpea is considered sensitive to salinity, but the salinity resistance of chickpea germplasm has rarely been explored. This study aimed to: (i) determine whether there is consistent genetic variation for salinity resistance in the chickpea mini-core and reference collections; (ii) determine whether the range of salinity resistance is similar across two of the key soil types on which chickpea is grown; (iii) assess the strength of the relationship between the yield under saline conditions and that under non-saline conditions; and (iv) test whether salinity resistance is related to differences in seed set under saline conditions across soils and seasons. The seed yield of 265 chickpea genotypes in 2005-06 and 294 cultivated genotypes of the reference set in 2007-08 were measured. This included 211 accessions of the mini-core collection of chickpea germplasm from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). The experiments were conducted in a partly-controlled environment using a Vertisol soil in 2005-06 and an Alfisol soil in 2007-08, with or without 80 mM sodium chloride (NaCl) added prior to planting. In a separate experiment in 2006-07, 108 genotypes (common across 2005-06 and 2007-08 evaluations) were grown under saline (80 mM NaCl) and non-saline conditions in a Vertisol and an Alfisol soil. In 2005-06 in the Vertisol and 2007-08 in the Alfisol, salinity delayed flowering and maturity, and reduced both shoot biomass and seed yield at maturity. There was a large variation in seed yield among the genotypes in the saline pots, and a small genotype by environment interaction for grain yield in both soil types. The non-saline control yields explained only 12 to 15% of the variation of the saline yields indicating that evaluation for salinity resistance needs to be conducted under saline conditions. The reduction in yield in the saline soil compared with the non-saline soil was more severe in the Alfisol than in the Vertisol, but rank order was similar in both soil types with a few exceptions. Yield reductions due to salinity were closely associated with fewer

pods and seeds per pot (61 to 91%) and to lesser extent from less plant biomass (12 to 27%), but not seed size. Groups of consistently salinity resistant genotypes and the ones specifically resistant in Vertisols were identified for use as donor sources for crossing with existing chickpea cultivars.

## **Introduction**

Worldwide about 100 M ha of arable land is affected by soil salinity and the area is expanding (Ghassemi et al. 1995). Chickpea as a crop species is sensitive to salinity (Flowers et al. 2010). The decline in the area sown to chickpea in traditional chickpea-growing areas of northern India and the Indo-Gangetic Plain (Gowda et al. 2009) is partly due to increased soil salinity and increased use of brackish water for irrigation. If this decline is to be reversed, then resistance of existing chickpea varieties to salinity needs to be improved. Since management options are often too expensive for small-holder farmers to adopt, breeding and selection of salinity-resistant varieties remains a more practical and immediate option. In Australia, chickpea is an important crop on neutral-to-alkaline Vertisol and Alfisol soils where it is one of the few crop legume options available for rotation with wheat. In many areas of Australia secondary salinity is an increasing problem, particularly on soils suitable for growing chickpea.

Until recently, little genetic variation for salinity resistance had been observed in chickpea (Saxena 1984; Dua 1992; Johansen et al. 1990). However, Vadez et al. (2007) found a six-fold range in seed yield of 263 chickpea genotypes grown in an artificially-salinized Vertisol watered to field capacity with 80 mM sodium chloride. Vertisols are usually high in organic matter and have a high cation exchange capacity that may reduce the effectiveness of the salt treatment. Chickpeas are also widely grown on Alfisol soils so it is important to assess whether the germplasm previously found to perform well in a salinized Vertisol also performs well in a salinized Alfisol if the germplasm is to be used in breeding programs for a wide range of soil types. Further, Vadez et al (2007) showed that the seed yield under salinity stress in chickpea was closely associated with time to flowering and to the seed yield under non-saline conditions. The study by Vadez et al. (2007) was conducted in the short-season environment of south India and this chickpea was planted late. This may have overemphasized the importance of phenology and the strength of the relationship between yield under saline and non-saline conditions.

Whether the relationship between seed yield under saline and non-saline conditions is robust is important when developing a breeding strategy, as selection for yield in non-saline conditions would be an easier option than selection under saline conditions, as previously asserted by Richards (1983). Here, we re-examine these relationships by having different soils and sowing at the regular date.

Several reports have shown that the resistance to salinity in chickpea is related to the resistance of reproduction (Mamo et al., 1996; Katerji et al., 2001). Salinity resistance indeed had been shown to be associated with the capacity to maintain a large number of filled pods, rather than to the capacity to grow under salt stress (Vadez et al., 2007), indicating that salt stress may have a deleterious effect on flower production and abortion and pod production and abortion. Yet, reproductive success may have been conditioned by the late-sown conditions in which the previous work was carried out (Vadez et al., 2007) and needs to be validated with sowing at the normal sowing time.

As salinity is likely to be an increasing problem in a warming and drying world, especially for relatively sensitive crops such as chickpea, it is important to make sources of resistance available to the breeding community by systematically screening a representative set of germplasm. To date, only the mini-core collection of chickpea germplasm has been evaluated for salinity resistance (Vadez et al., 2007). This mini-core collection is based on morphological and agronomic traits (Upadhyaya and Ortiz 2001) and not a systematic screening for diversity of molecular markers. More recently, a reference collection of chickpea has been assembled using marker data from 50 SSR markers screened in over 3,000 genotypes (Upadhyaya et al., 2006). Although the reference collection includes all the germplasm in the mini-core collection, 89 additional entries of cultivated chickpea with additional molecular variability have been identified (Upadhyaya et al. 2008).

Thus, the present study was initiated to determine the salinity resistance of a wide range of germplasm in the two soil types in which chickpea is widely grown. Specifically, the aims of the present study were: (i) to determine whether the range of salinity resistance is similar across two of the key soil types, a Vertisol and an Alfisol, on which chickpea is grown; (ii) to assess the strength of the relationship between the yield under saline conditions and that under non-saline conditions; (iii) to test whether salinity resistance is related to differences in seed set under saline conditions across soils and seasons; and (iv) to test whether the additional genotypes in the reference collection add new sources of variation in salinity resistance, and to provide a robust list of highly-contrasting lines with salinity resistance for use by breeders.

## **Materials and Methods**

### **Plant growth, treatment conditions, sowing dates and genetic material**

Plants were grown in pots filled with soil that was either left untreated (non-saline treatment) or treated with NaCl (saline treatment) in an open-air facility that was protected from rain by a movable rain-out shelter. Experiments were undertaken, in three years at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) headquarters in Patancheru, Andhra Pradesh, India (17°32' N, 78°16' E, altitude: 546 m above sea level) with sowing on 11 November 2005, 31 October 2006 and 25 October 2007, and harvested when mature or before the second week of March. Maximum temperatures in the growing season ranged from 25.2–35.4°C in 2005–06, 24.1–32.7°C in 2006–07 and 26.5–33.8°C in 2007–08, and minimum temperatures ranged from 6.7–21.6°C in 2005–06, 8.6–21.7°C in 2006–07 and 7.3–22.3°C in 2007–08.

The pots (27 cm diameter), containing 8.0 kg of Vertisol in 2005–06 and 9 kg of an Alfisol in 2007–08, were buried in plots such that the pot rim and the outside soil surface were at the same level to avoid direct solar heating of the pots. In 2006–07, the experiment included both

soil types. The Vertisol (pH = 8.1, cation exchange capacity (CEC):clay ratio = 0.87, EC = 0.1 mM) and the Alfisol (pH = 6.9, CEC:clay ratio = 0.29, EC = 0.1 mM (El Swaify et al. 1985) taken from the top 10 cm of soil at the ICRISAT farm, were fertilized with di-ammonium phosphate (DAP) at a rate of 300 mg kg<sup>-1</sup> soil. In 2005-06, half of the pots were artificially salinized by applying a dose of 1.17 g NaCl kg<sup>-1</sup> (Vertisol) and in 2007-08 half of the pots were salinized by applying 0.94 g NaCl kg<sup>-1</sup> (Alfisol), equivalent to applying a 80 mM solution of NaCl in sufficient volume (1.875 L and 1.80 L, respectively) to wet the Vertisol and Alfisol to field capacity (25% and 20% w/w, respectively). In 2006-07 a third of the pots were filled with Vertisol soil salinized as in 2005-06, while a third were filled with Alfisol soil salinized as in 2007-08; the remaining third were filled with Alfisol soil that was not salinized: These and the remaining pots in 2005-06 and 2007-08 received tap water containing no significant amount of NaCl in the same quantities to bring them to field capacity

The saline treatment was applied as two half doses at sowing and 12 days after sowing to more realistically represent a field situation than a single application. After salt application and for the remaining crop cycle, pots were watered with tap water and maintained close to a range of 60-90% field capacity (determined gravimetrically) to avoid an increase in the salt concentration in the soil solution. The base of the pots of the saline treatment was sealed to avoid salt leakage, while the pots of the non-saline treatment had holes to allow drainage. Over-watering of all pots was avoided. This method has had consistently good results in chickpea and other crops (Vadez et al. 2007; Srivastava et al. 2006; 2008).

In all three years, six seeds were planted in each pot and at 12 days after sowing thinned to four plants per pot. The experiments were planted in a 18×15 alpha lattice (incomplete block design) in 2005-06, in a 21×14 alpha lattice in 2007-08 with two factors (saline and non-saline), in a 18×6 alpha lattice (incomplete block design) in 2006-07 with three factors and three replications in all three seasons. In 2005-06, 265 entries were tested, including 211 accessions

from the ICRISAT mini-core collection (Upadhyaya and Ortiz 2001) and 54 accessions including popular cultivars, breeding lines reported as resistant to salinity/sodicity (Dua and Sharma 1995), and one cultivar previously released by the Central Soil Salinity Research Institute (CSSRI), Karnal, India as salinity resistant (CSG 8962). The mini-core accessions used for the salinity-resistance evaluation came primarily from India and Iran, but a total of 24 countries were represented in the collection (Upadhyaya et al. 2001). Out of the 265 accessions, 60 were kabuli type, 197 desi type and 8 were intermediate. In 2006-07, 108 of the 265 genotypes, used in 2005-06 were used; 80 with the highest yields and 28 with the lowest yields in the saline treatment. In 2007-08, the cultivated entries of the reference collection were tested, which included the 211 accessions from the ICRISAT mini-core collection plus 83 additional cultivated chickpea accessions (Upadhyaya et al. 2008) (n=294). All tested entries are hereafter referred to as genotypes.

### **Measurements**

Days to 50% flowering, days to maturity, shoot biomass at maturity ( $\text{g pot}^{-1}$ ) including pods but not most of the leaflets that had fallen to the ground by maturity, seed yield at maturity ( $\text{g pot}^{-1}$ ), pod number  $\text{pot}^{-1}$ , seed number  $\text{pod}^{-1}$  and 100-seed weight were measured in each year. The shoot, seed yield and pod numbers are presented on a per pot basis as in previous studies. Weight or number per pot was found to be a more realistic measure of performance than weight or number per plant in the rare cases when one or two plants failed to grow.

### **Statistical approach to test the genotypic and genotype by environment (G×E) effects on seed yield under salinity**

Data from individual experiments were analyzed using the following linear additive mixed effects model (Breslow and Clayton 1993):

$$Y_{ijk} = \mu + r_i + b_{ij} + g_k + e_{ijk}$$



where  $y_{ijk}$  is the observation recorded on genotype  $k$  in an incomplete block  $j$  of replicate  $i$ ,  $\mu$  is the general mean,  $r_i$  is the effect of replicate  $i$ ,  $b_j$  is the effect of block  $j$  within replicate  $i$ ,  $g_k$  is the effect of genotype  $k$ , and  $e_{ijk}$  is the effect of the plot. The general mean  $\mu$  and replicate effect  $r_i$  were considered as fixed effects. The block effect  $b_j$ , genotype effect  $g_k$ , and plot effect  $e_{ijk}$ , were assumed as random effects each with mean zero and constant variances  $\sigma_b^2$ ,  $\sigma_g^2$  and  $\sigma_e^2$  respectively. Using the above model, the statistical procedure of residual maximum likelihood (ReML) (Harville 1977) was employed to obtain unbiased estimates of variance components  $\sigma_b^2$ ,  $\sigma_g^2$  and  $\sigma_e^2$ , and the best linear unbiased predicted means of genotypes (BLUPs) as the performance of germplasm accessions. Heritability was estimated as  $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2)$ . The significance of genetic variability among accessions was assessed from the standard error (SE) of the estimate of genetic variance  $\sigma_g^2$ , assuming the ratio  $\sigma_g^2 / SE(\sigma_g^2)$  to follow normal distribution asymptotically. The first year data was analyzed without considering the block effects.

The above model was extended for over-year analysis of traits recorded in the two years 2005-06 and 2007-08 with a large number (217) of common genotypes, assuming year as fixed, with genotype x environment interaction (GxE) being a random effect assumed to have a mean of zero and constant variance  $\sigma_{gE}^2$ . The significance of GxE was assessed in a manner similar to that of  $\sigma_g^2$ . The significance of the fixed effect of the year was assessed using the Wald statistic that asymptotically follows a  $\chi^2$  distribution and is akin to the F-test in the traditional ANOVA.

As seed yield of germplasm accessions under salinity across years had a significant interaction, their BLUPs were further grouped into various response groups for salt reaction by a hierarchical cluster analysis using the linkage method with incremental sum of squares (Ward 1963). All statistical analyses were carried out using Genstat, Release 10.1 (Payne 2002).

## **Results**

### **Screening for salt resistance**

#### **Seed yield and biomass accumulation under salinity**

In 2005-06 and 2007-08, plant growth in the non-saline treatment achieved in the pots were equivalent to 3-4 t ha<sup>-1</sup> of shoot biomass and 1.5-2.0 t ha<sup>-1</sup> seed yield, values similar to those in the field in the local environment. The saline treatment reduced overall shoot biomass at maturity by 40-60% and seed yield by 57-77% (Table 1). Two- to three-fold variation for shoot biomass and over six-fold variation for seed yield was observed within the chickpea genotypes (Table 1). The reduction in shoot biomass and seed yield was greater in 2007-08 when chickpea genotypes were grown in the Alfisol than in 2005-06 when these were grown in the Vertisol, but there was significant variation among genotypes, regardless of the soil type (Table 1). The heritability indices for shoot biomass and seed yield under salinity were 0.51 and 0.58, respectively, in the Vertisol in 2005-06 and 0.10 and 0.21, respectively, in the Alfisol in 2007-08.

INSERT Table 1 ABOUT HERE

#### **Phenological changes with salinity**

Highly significant and large variation was observed among chickpea genotypes for time to 50% flowering and maturity. Salinity delayed the time to 50% flowering by 7 to 8 days in the Vertisol in 2005-06 and 14 days in the Alfisol in 2007-08 (data not shown). Under saline conditions, the mean 50% flowering time of the genotypes was at 58 days after sowing (DAS) in the Vertisol (2005-06) and 65 DAS in the Alfisol (2007-08). The range in time to 50% flowering among the genotypes was 27 to 79 DAS in the Vertisol and 33 to 98 DAS in the Alfisol. The heritability values for time to flowering under salinity were high and ranged from 0.72 to 0.85 across soil types, similar to those observed under non-saline conditions. The saline

treatment delayed the time to maturity by 9 days only in the Alfisol (2007-08). Thus salinity increased the vegetative period of growth, but reduced the reproductive period of growth. The heritability indices for the time to maturity under salinity were 0.67 and 0.73 in both soil types.

In the Vertisol in 2005-06, seed yield under salinity increased with time to 50% flowering until 50 DAS and then decreased (Fig.1). This curvilinear response explained 29% of the variation in grain yield under salinity. In the Alfisol in 2007-08, seed yield among genotypes under salinity decreased as the time to 50% flowering increased from 35 to 100 DAS (Fig.1).

INSERT FIGURE 1 ABOUT HERE

### **Yield components under salinity**

Yield components, such as pod number, seed number, seeds pod<sup>-1</sup> and 100-seed weight, were all adversely affected by the saline treatment (Table 2). Pod number pot<sup>-1</sup> decreased by 52% in the Vertisol in 2005-06 and 69% in the Alfisol in 2007-08. In the non-saline pots, less than 20% of pods on an average had two seeds pod<sup>-1</sup> and salinity reduced the number of seeds pod<sup>-1</sup> by 3% and 7% in 2005-06 and 2007-08, so that seed number pot<sup>-1</sup> decreased by 55% in the Vertisol in 2005-06 and 71% in the Alfisol in 2007-08. Salinity also reduced 100-seed weight by 6% in Vertisol in 2005-06 and by 32% in the Alfisol in 2007-08. Thus the pod and seed numbers per plant were the most adversely affected yield components from the salinity treatment, while seed size was affected more in 2007-08 when the chickpeas were grown in Alfisol and salinity had a greater effect on yield. It is notable that the heritability of yield components was lowest (0.27 for pod number pot<sup>-1</sup> and 0.53 for 100-seed weight) when salinity stress was the severest, i.e. 2007-2008 in the Alfisol.

INSERT Table 2 ABOUT HERE

### **Relationship of yield with biomass and yield components.**

In saline soil the seed yield of the genotypes was poorly associated with the shoot biomass at maturity, the association only explaining about 12% of the variation in the Vertisol (2005-06) and 27% in the Alfisol in 2007-08 (Fig 2). However, the seed yield in the saline soil was closely and positively correlated with pod number, with pod number accounting for 61% of the variation in seed yield in 2005-06 and 91% in 2007-08. Seeds pod<sup>-1</sup> ( $r^2 = 0$  to 2%) and 100-seed weight ( $r^2 = 0$  to 4%) were not associated with seed yield pot<sup>-1</sup> under saline conditions (data not shown).

INSERT FIGURE 2 ABOUT HERE

### **Genotype by salinity level and genotype by year/soil type interactions**

The interaction between the genotypes and the saline treatments ( $\sigma_{gE}^2$ ) for seed yield was significant in both soil types/years [Vertisol (2005-06) = 5.81, SE 1.41; Alfisol (2007-08) = 5.79, SE 2.38], indicating that the ranking of seed yield of genotypes under non-saline control differed from that of the ranking under salinity. Further, the relationship of seed yield of the genotypes in the saline soil with seed yield of the same genotypes in non-saline soil was positive and linear in both the Vertisol in 2005-06 and Alfisol in 2007-08, but the relationship explained only 12 to 15 % of the variation (Fig. 3).

A pooled analysis of the seed yield of common accessions (n=217) in the saline treatment across the two years/soil types showed large genotypic variation ( $\sigma_g^2 = 5.04$ , SE 0.99) and a significant, but considerably smaller, genotype by year/soil type interaction ( $\sigma_{gE}^2 = 2.16$ , SE 0.88).

INSERT FIGURE 3 ABOUT HERE

### **Effect of soil type on salinity responses in the same year**

As the previous comparisons of the genotypic responses to salinity between those grown in an Alfisol and those grown in a Vertisol were made in different years, in 2006-07 a limited set of the genotypes (n=108) was compared in the two soil types in the same year. There was little correlation ( $r^2 = 0.02$ ) among genotypes for their shoot biomass in the saline Alfisol and saline Vertisol soils, but a better correlation was noticed ( $r^2 = 0.20$ ) between the seed yields in the two saline soils (Fig. 4). Salinity in the Alfisol significantly reduced the mean shoot biomass at maturity by 42%, but only by 6% in the saline Vertisol compared to the non-saline Alfisol. However, the seed yield was reduced by 67% in the saline Alfisol compared to 23% in saline Vertisol (data not shown), similar to the results obtained when the soil types were compared in different years. This correlation was even better when four of the genotypes (ICC 2580, ICC 12866, ICC 9942, ICC 32) that were high yielding in the Alfisol were excluded ( $r^2 = 0.30$ ). The soil type  $\times$  genotype interaction was large and significant for both the shoot biomass ( $21.2 \pm 8.4$ ) and seed yield ( $11.6 \pm 2.5$ ). However, a significant rank correlation between the means in the saline Vertisol and in the saline Alfisol ( $r = 0.49^{***}$ ) showed that the interaction was a non-cross over type. However, the rank correlation between the yield of a genotype in the non-saline Alfisol and saline Alfisol was not significant ( $r = 0.17^{NS}$ ). We conclude that the sensitive genotypes in the Vertisol were also sensitive in an Alfisol, whereas the tolerant ones in the Vertisol can have varying resistance to salinity in the Alfisol soil. The four genotypes that yielded well in the Alfisol (Fig. 4) were also ones that flowered at the optimum flowering time (50 days) for this location (Fig. 1). A regression of the genotypic means from the saline Alfisol in 2006-07 with that of 2007-08 explained 30% of the variation, while the means of saline Vertisol in 2006-07 with the same soil in 2005-06 explained 58% of the variation.

INSERT Fig 4 ABOUT HERE

### **Identification of genotypes varying in seed yield under salinity**

As there was a significant interaction between genotypes and soils/years in the saline treatment, the seed yield of the genotypes for the studies in the two years 2005-06 and 2007-08 were grouped using best linear unbiased predictors (BLUPs) for seed yield by a hierarchical cluster analysis (using Ward's incremental sum of squares method). This analysis yielded at a 75% similarity level for five major groups (Table 3). The analysis did not include the data from 2006-07 as the number of genotypes in that year was much smaller than in the other two years. These groups were: (i) consistently highly resistant (n=12, listed in Table 4); (ii) highly resistant only in the Vertisol (n=46, listed in Table 5); (iii) consistently resistant (n= 31, listed in Table 6); (iv) resistant only in the Vertisol (n=65); and (v) consistently highly sensitive (n=63, listed in Table 7). Once again it is clear from the BLUPs that compared to the highly resistant group, the highly sensitive ones are relatively late in flowering, have the same or slightly higher shoot biomass, and have about a 50% reduction in seed yield (Table 3).

INSERT Table 3, Table 4, Table 5, Table 6 and Table 7 ABOUT HERE

In addition to the 211 genotypes in the mini-core collection, 54 additional genotypes, mostly popular cultivars and breeding lines, were evaluated in 2005-06 for yield under salinity. In this year, the saline yield of CSG 8962, the salt tolerant check, was 9.2 g pot<sup>-1</sup> compared to the trial mean of 8.8 g pot<sup>-1</sup>. Several other released cultivars such as KAK 2, ICCV 10, JG 74, Vijay, WR 315, Annigeri, K 850, L550, JG 11, JG 6 and, C 235, as well as breeding lines such as ICC 4953, ICCL 87322, ICCL 85222 and ICCL 82108 yielded significantly better than the overall mean. Of the 83 genotypes from the reference collection that were added in 2007-08, 18 yielded better than the overall mean, but only two accessions ICC 15785 and ICC 16654 produced significantly greater seed yield in the saline treatment than the overall mean in this

trial (2007-08), while ICC 3892, ICC 4853, ICC 5221, ICC 9712, ICC 10018 and ICC 12324 had substantially higher yields than CSG 8962. The sensitive genotypes in the reference collection that yielded less than 4 g pot<sup>-1</sup> in the saline treatment were ICC 3410, ICC 3582, ICC 4093, ICC 9702, ICC 10466, ICC 10685, ICC 12379, ICC 15614 and IG 5909.

## **Discussion**

This study with experiments conducted over three years has demonstrated that there is wide variation in chickpea genotypes for salinity resistance and that 12 genotypes were highly resistant in both a Vertisol and an Alfisol soil. One accession, ICC 9942, had the highest and most consistent seed yield in both soil types (across years) as well as in the previously-reported study (Vadez et al 2007). Indeed, this study is consistent with the previous work of Vadez et al. (2007) in confirming the resistance of many of the genotypes. Similar consistency could also be seen with the sensitive genotypes (Table 6). With salinity being an increasingly important issue throughout the world, particularly when saline groundwater is used for irrigation or there is increasing secondary salinization, identification of genotypes with high salinity resistance is invaluable. In India the only genotype released solely for salinity/sodicity resistance/tolerance is CSG 8962 which is a medium-duration cultivar and unlikely to perform well in the short-duration environment of this study. Moreover, it only was highly resistant in the Vertisol soil. Of the 265 genotypes evaluated in 2005-06, about 20% were in the consistently highly tolerance group. About one third of these highly resistant entries were either long-standing cultivars for short- (Annigeri and JG 62), medium- (JG 11) and long-duration (L 550) environments or were newly-bred desi and kabuli cultivars such as ICCL 85222, ICCL 87322, ICCV 10, ICCV 96836 or Vijay. The inclusion of 83 cultivated genotypes from the reference collection in 2007-08 in addition to the 211 genotypes from the mini-core collection identified 18 genotypes that yielded significantly better than the overall mean in the Alfisol soil. The one accession, ICC 9942, that had the highest and most consistent seed yield across all years and soil types is unfortunately a

medium-duration, small-seeded (10-12.5 g per 100-seed weight) desi-type with a wrinkled seed surface that is not desirable in the market for whole-seed consumption. However, it will make an excellent parent in a breeding program for salt resistance. Genotypes with acceptable seed characteristics and a good level of salinity resistance, in desi types such as ICC 1431 and K 850 and kabuli types such as L 550 and ICCV 95311, could also be useful additional salt-resistant parents. Identification of genotypes that are tolerant across various stresses could lead to more rapid progress in breeding. However, the genotype ICC 8261, with a reported large root system (Kashiwagi et al. 2005) and putative drought resistance, was salt-sensitive (Table 6). This suggests that a strong root system does not contribute to salinity resistance or that soil salinity does not allow normal growth of the roots, something that is worthy of future investigation. ICC 10885, one of the most drought-sensitive genotypes (Saxena 2003), also was sensitive to salinity, while two genotypes putatively differing in drought resistance (one was early flowering and escaped drought compared to the late flowering, drought sensitive genotype) had similar salinity resistance (Katerji et al. 2001).

The saline treatment (80 mM NaCl) reduced seed yields more in the Alfisol than in the Vertisol, suggesting that soil type plays a major role in the effect of the salinity treatment on yield. Also the reduction in shoot biomass was greater in the Alfisol than in the Vertisol, indicating the level of salinity experienced by the plants in the Alfisol was more severe than in the Vertisol with the same level of salt application. An increased electrical conductivity in the soil solution in the Alfisol compared to the Vertisol is likely due to the low cation exchange capacity as well as the relatively low level of organic matter of the Alfisol. Nevertheless, despite the lower yield in the Alfisol, the genotypic rankings to a large extent were maintained as in the Vertisol. A few genotypes, such as ICC 2580, ICC 9942 and ICC 12866 desi types and the kabuli type ICC 32, deviated from the general pattern to give a higher yield in the Alfisol than the Vertisol, indicating that these genotypes are well adapted to saline Alfisol soils. The



large genotypic variation in shoot biomass exhibited in the saline Vertisol was much smaller in the Alfisol, while yields in both the non-saline soils explained only a small part (12 to 15%) of the saline yields (Fig. 3). This suggests that the screening methodology used in the study based on seed yield and seed/pod number is reliable across soil types and can be used to select parents for future salt resistance breeding in chickpea, but evaluation for adaptation to saline environments needs to be conducted in targeted stress environments for success. In light of the large G×E interaction, the current effort towards molecular marker-assisted breeding to enhance salt resistance of chickpea (Flowers et al. 2010) is appropriate. Also the confounding effect of flowering time (crop duration) on seed yield also needs to be understood and removed (Vadez et al. 2007) while making selections for salinity resistance.

In comparison with seed yield in the non-saline treatment, the seed yield in the saline treatment was affected in two ways: a direct reduction in plant size and a reduction in the reproductive components and subsequent partitioning. Mean shoot biomass at maturity decreased by 40 and 60% and seed yield decreased by 57 and 77% in 2005-06 and 2007-08, respectively (Table 1). This suggests that the yield reduction was a result of less biomass accumulation and therefore reproductive sites and more importantly from a reduction in the flowers that produced a pod and seed as also had been the case with common bean (*Phaseolus vulgaris* L.) or mungbean (*Vigna radiata* (L.) Wilczek) (Bourgault et al. 2010) and cowpea (Praxedes et al 2010). Vadez et al. (2007) previously showed in chickpea that genotypic yield differences under saline conditions were not related to genotypic differences in biomass accumulation. In that experiment, the ratio of yield (yield in the saline treatment/yield in the non-saline treatment) was closely correlated with the ratio in pod number, which also helps to explain 76 and 90% of variation in this study in 2005-06 and 2007-08 respectively. Seed size (100-seed weight) was reduced by salinity, but to a much lesser degree (5 and 33% in 2005-06

and 2007-08, respectively) (Fig 2). Seed size is often maintained under stress conditions that reduce other yield components (Turner et al. 2001).

As there is generally only a small proportion of pods that have more than one seed, pod number plant<sup>-1</sup> was the yield components most affected by salinity. Pod number has been identified as the major yield component affected by many production constraints (Leport et al. 1999; Whish et al. 2007). In water-limited environments, chickpea produced fewer pod-bearing sites (nodes) and also had a greater number of flowers and pods that aborted (Leport et al. 2006; Fang et al. 2010). This study has shown that genotypes with fewer pods in the non-saline treatment also produced fewer pods in the saline treatment, while genotypes that had more pods in the non-saline treatment exhibited a greater range of variation in the saline treatment (Fig. 5), providing an opportunity for selection of genotypes with a greater number of pods per plant under saline conditions. The current requirements of molecular genetics demand simple traits and high throughput phenotyping protocols capable of handling large number of entries at a given time. In the case of salinity resistance simple traits and protocols are not available and selection for yield under saline conditions is required. Other traits related to seedling or shoot biomass productivity at flowering (Serraj et al. 2004; Maliro et al. 2008) are inadequate as surrogates for final yield (Vadez et al. 2007; Bourgault et al. 2010). The strong association between pod number and seed yield under saline conditions suggests that selection for high pod number under saline conditions may be a possible alternative in breeding programs, especially since the heritability for pod number and seed number was higher than for seed yield.

## **Conclusions**

This study has shown wide genotypic variation for salinity resistance in the chickpea mini-core and reference collections and that the salinity resistance observed in an Alfisol soil was similar in the majority of genotypes to that in a Vertisol soil. A group of resistant genotypes and a

group of sensitive genotypes in terms of seed yield in the saline treatment has been identified and genotypes suitable as parents for introgression of salinity resistance have been provided. Reduction in seed yield in the saline treatment was primarily associated with fewer pods and seeds per plant, whereas seed size (100-seed weight) was less affected, and shoot biomass did not explain the genotypic differences in seed yield.

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**Table 1** Overall mean, range of best linear unbiased predicted means of genotypes (BLUPs) with the standard error of difference (SED) in parenthesis, genetic variance ( $\sigma^2_g$ ) with its standard error in parenthesis, for shoot biomass and seed yield at maturity of 265 chickpea genotypes (211 from the mini-core collection) in a saline and non-saline Vertisol soil in 2005-06 and 294 chickpea genotypes in a saline and non-saline Alfisol soil in 2007-08.

Season/ Environment	Trial mean	Range of predicted Means (SED)	$\sigma^2_g$ (SE)
Shoot biomass (g pot <sup>-1</sup> )			
Vertisol			
Non-saline	53.6	39.2 – 69.8 (6.94)	48.1 (8.8)
Saline	32.2	16.5 – 51.4 (4.04)	33.6 (3.9)
Alfisol			
Non-saline	55.8	49.1 – 70.4 (8.23)	46.8 (15.7)
Saline	22.4	17.3 – 32.4 (5.49)	19.9 (7.7)
Seed yield (g pot <sup>-1</sup> )			
Vertisol			
Non-saline	20.3	11.5 – 29.1 (4.38)	18.31 (3.53)
Saline	8.80	1.8 – 15.0 (1.56)	6.29 (0.68)
Alfisol			
Non-saline	18.9	10.0 – 29.4 (5.57)	29.61 (5.72)
Saline	4.3	2.4 – 15.7 (2.93)	7.75 (1.54)



**Table 2** Overall means, range of best linear unbiased predicted means of genotypes (BLUPs) with the standard error of difference (SED) in parenthesis, genetic variance ( $\sigma^2_g$ ) with its standard error in parenthesis for pod number per pot, seed number per pot, seeds per pod and 100-seed weight of 265 chickpea genotypes (211 from the mini-core collection) in a saline and non-saline Vertisol soil in 2005-06 and 294 chickpea genotypes in a saline and non-saline Alfisol soil in 2007-08.

Season/ Environment	Trial mean	Range of predicted means (SED)	$\sigma^2_g$ (SE)
Pod number (pot <sup>-1</sup> )			
Vertisol			
Non-saline	101.4	43.5 – 185.4 (28.6)	1067 (154)
Saline	48.4	6.7 – 121.3 (9.8)	361.6 (36.1)
Alfisol			
Non-saline	107.5	38.1 – 201.3 (36.8)	1930 (253)
Saline	33.5	15.7 – 138.5 (22.2)	526 (86)
Seeds pod <sup>-1</sup>			
Vertisol			
Non-saline	1.18	0.90 – 1.49 (0.11)	0.0171 (0.0025)
Saline	1.15	0.94 – 1.51 (0.13)	0.0144 (0.0022)
Alfisol			
Non-saline	1.19	1.07 – 1.78 (0.14)	0.0109 (0.0038)
Saline	1.11	0.98 – 1.31 (0.12)	0.0085 (0.0039)
100-seed weight (g)			
Vertisol			
Non-saline	19.5	11.6 – 44.5 (2.43)	46.93 (4.32)
Saline	18.3	8.9 – 48.8 (3.12)	47.68 (4.36)
Alfisol			
Non-saline	17.4	8.8 – 38.3 (3.75)	44.88 (3.95)
Saline	11.8	6.8 – 27.5 (3.74)	14.80 (1.74)

Table 3. The overall best linear unbiased predicted means of genotypes (BLUPS) for days to 50% flowering, shoot biomass and seed yield of various salinity response groups (based on saline seed yield) in a Vertisol soil in 2005-06 and an Alfisol soil in 2007-08.

Group type (No. of genotypes)	Soil	Days to 50% flowering	Shoot biomass (g pot <sup>-1</sup> )	Seed yield (g pot <sup>-1</sup> )
Consistently highly resistant (n=12)	Vertisol	56	30.5	10.5
	Alfisol	54	26.3	10.3
Highly resistant only in Vertisol (n=46)	Vertisol	57	33.1	11.0
	Alfisol	59	20.9	4.2
Consistently resistant (n=31)	Vertisol	55	31.3	9.7
	Alfisol	56	23.3	6.5
Resistant only in Vertisol (n=65)	Vertisol	59	31.9	9.1
	Alfisol	62	21.3	4.2
Consistently highly sensitive (n=63)	Vertisol	64	35.6	5.8
	Alfisol	71	21.9	3.4

**Table 4.** Best linear unbiased predicted means of genotypes (BLUPs) of days to 50% flowering, shoot biomass and seed yield for the group of consistently highly resistant accessions (n=12) in a Vertisol soil in 2005-06 and an Alfisol soil in 2007-08.

Accession	Vertisol			Alfisol		
	Days to 50% flowering	Shoot biomass (g pot <sup>-1</sup> )	Seed yield (g pot <sup>-1</sup> )	Days to 50% flowering	Shoot biomass (g pot <sup>-1</sup> )	Seed yield (g pot <sup>-1</sup> )
ICC 9942	57	31.9	12.8	54	32.4	15.7
ICC 6279	45	28.4	11.1	43	25.5	11.1
ICC 11121	60	30.5	11.0	60	26.9	10.7
ICC 456	61	24.4	10.7	55	26.5	11.3
ICC 14799	60	35.8	10.6	60	25.1	8.6
ICC 1710	65	32.0	10.4	67	25.7	8.5
ICC 791	63	33.4	10.3	68	25.4	9.0
ICCV 95311	46	28.2	10.2	45	25.7	9.1
ICC 12155	60	32.1	10.1	50	26.0	12.4
ICC 4918	43	30.8	9.9	50	25.5	9.9
ICC 3325	63	28.0	9.7	54	27.1	9.7
ICC 5613	52	30.8	9.6	46	23.5	7.8

**Table 5.** Best linear unbiased predicted means of genotypes (BLUPs) of days to 50% flowering, shoot biomass and seed yield for the group of highly resistant only in the Vertisol genotypes (n=46) in a Vertisol soil in 2005-06 and an Alfisol soil in 2007-08.

Accession	Vertisol			Alfisol		
	Days to 50% flowering	Shoot biomass (g pot <sup>-1</sup> )	Seed yield (g pot <sup>-1</sup> )	Days to 50% flowering	Shoot biomass (g pot <sup>-1</sup> )	Seed yield (g pot <sup>-1</sup> )
ICC 12824	52	37.0	15.0	48	20.1	4.6
ICCV 10	56	35.3	13.9	57	21.1	5.0
ICC 7819	63	47.0	13.2	69	21.9	4.0
ICC 8950	59	31.3	12.5	65	20.5	4.4
ICC 10399	53	36.0	12.3	51	20.5	5.2
ICC 14669	52	33.4	12.1	48	19.7	4.4
ICC 15868	59	33.8	11.9	70	20.0	4.9
ICC 6816	59	32.4	11.7	62	19.3	4.1
ICC 5878	48	31.5	11.7	42	19.6	5.0
ICC 7554	61	39.6	11.6	73	21.5	3.4
ICC 1083	53	27.9	11.6	51	20.0	5.2
ICC 283	55	32.1	11.6	49	21.3	5.7
ICC 8621	56	30.2	11.5	56	20.2	4.9
ICC 9755	47	31.6	11.4	60	21.6	4.9
ICC 1230	54	34.7	11.4	51	22.1	4.3
ICC 4495	60	34.7	11.3	60	21.5	5.3
ICC 14778	62	32.8	11.3	66	19.1	2.4
ICC 12028	61	37.5	11.2	71	20.1	3.6
ICC 1431	62	32.3	11.2	68	21.5	3.8
ICC 10945	54	30.7	11.1	54	20.0	3.8
ICC 7272	62	41.5	11.1	62	23.7	3.4
ICC 74411	53	29.3	11.0	55	19.7	4.1
ICC 95	59	34.0	11.0	57	20.4	4.3
ICC 11944	65	31.9	11.0	60	21.5	4.6
ICC 1180	63	38.5	10.9	79	20.1	3.8
ICC 11378	66	34.3	10.8	69	27.5	2.9
ICC 2580	58	31.3	10.8	53	20.2	4.6
ICC 5879	49	28.7	10.8	51	24.5	3.2
ICC 15888	57	32.7	10.8	57	20.7	4.5
ICC 14831	60	34.7	10.8	60	20.7	4.1
ICC 7184	62	38.6	10.5	67	18.4	2.8
ICC 1715	72	32.7	10.3	73	20.6	3.7
ICC 1356	59	30.1	10.3	58	20.5	4.6
ICC 5383	56	32.2	10.3	52	21.1	3.5
ICC 10393	36	30.2	10.2	45	20.4	4.5
ICC 13863	51	28.9	10.2	48	18.3	3.4
ICC 15294	49	36.8	10.2	66	20.9	2.9

ICC 13892	53	26.5	10.2	54	19.6	4.1
ICC 1164	61	28.0	10.2	69	20.2	4.1
ICCV 95423	53	28.1	9.9	58	20.7	4.4
ICC 16269	61	32.6	9.9	68	21.2	4.1
ICC 1397	63	33.5	9.8	75	21.3	4.4
ICC 1398	50	30.6	9.7	48	20.1	4.4
ICC 1392	61	29.3	9.5	64	21.2	4.2
ICC 9895	56	32.3	9.4	42	21.8	4.5
ICC 4841	68	32.6	9.4	80	22.7	4.6

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**Table 6.** Best linear unbiased predicted means of genotypes (BLUPs) of days to 50% flowering , shoot biomass and seed yield for the group of consistently resistant genotypes (n=31) in a Vertisol soil in 2005-06 and an Alfisol soil in 2007-08.

Accession	Vertisol			Alfisol		
	Days to 50% flowering	Shoot biomass (g pot <sup>-1</sup> )	Seed yield (g pot <sup>-1</sup> )	Days to 50% flowering	Shoot biomass (g pot <sup>-1</sup> )	Seed yield (g pot <sup>-1</sup> )
ICC 15606	58	34.1	12.0	49	23.5	8.0
ICC 11284	60	35.6	11.8	62	27.4	7.3
ICCL 82108	59	29.9	11.6	57	21.8	6.2
ICC 2263	57	33.1	11.2	57	22.7	7.2
ICC 5845	60	29.5	11.2	84	24.2	6.5
ICC 5639	55	31.7	11.1	49	23.0	7.1
ICC 867	58	32.5	11.1	50	23.8	7.0
ICC 14595	47	33.0	11.0	56	23.4	7.1
ICC 15264	46	33.3	10.9	54	23.7	6.2
ICC 7668	57	32.6	10.8	54	24.2	6.6
ICC 708	57	34.1	10.7	59	24.3	6.3
ICC 8384	59	32.3	10.6	51	22.9	6.6
ICC 12851	53	28.1	10.5	36	22.2	6.8
ICC 12866	45	27.1	10.5	39	20.7	5.8
ICC 14402	55	28.2	10.5	46	21.7	6.3
ICC 16915	51	33.9	10.3	51	22.4	6.9
ICC 3512	64	31.9	10.1	47	22.3	6.0
ICC 4872	33	27.5	9.2	33	21.5	6.4
ICC 4593	59	34.7	9.0	78	21.1	3.4
ICC 5434	49	31.7	9.0	40	20.3	5.8
ICC 1098	58	29.5	8.9	60	23.7	6.7
ICC 8058	46	33.2	8.8	70	25.2	5.7
ICC 13124	49	27.3	8.7	41	22.4	6.2
ICC 6874	57	27.0	8.4	59	21.5	6.3
ICC 12654	52	27.7	8.3	55	23.4	6.8
ICC 9848	41	31.9	8.2	54	24.5	6.3
ICC 6811	65	30.7	8.2	69	23.0	6.0
ICC 13524	57	33.2	7.8	64	24.6	6.5
ICC 15610	67	32.6	7.5	72	26.3	6.9
ICC 16524	59	31.0	7.2	58	23.0	6.2
ICC 762	73	32.1	4.7	83	28.8	8.5

**Table 7.** Best linear unbiased predicted means of genotypes (BLUPs) of days to 50% flowering, shoot biomass and seed yield for the group of consistently highly sensitive genotypes (n=63) in a Vertisol soil in 2005-06 and an Alfisol soil in 2007-08.

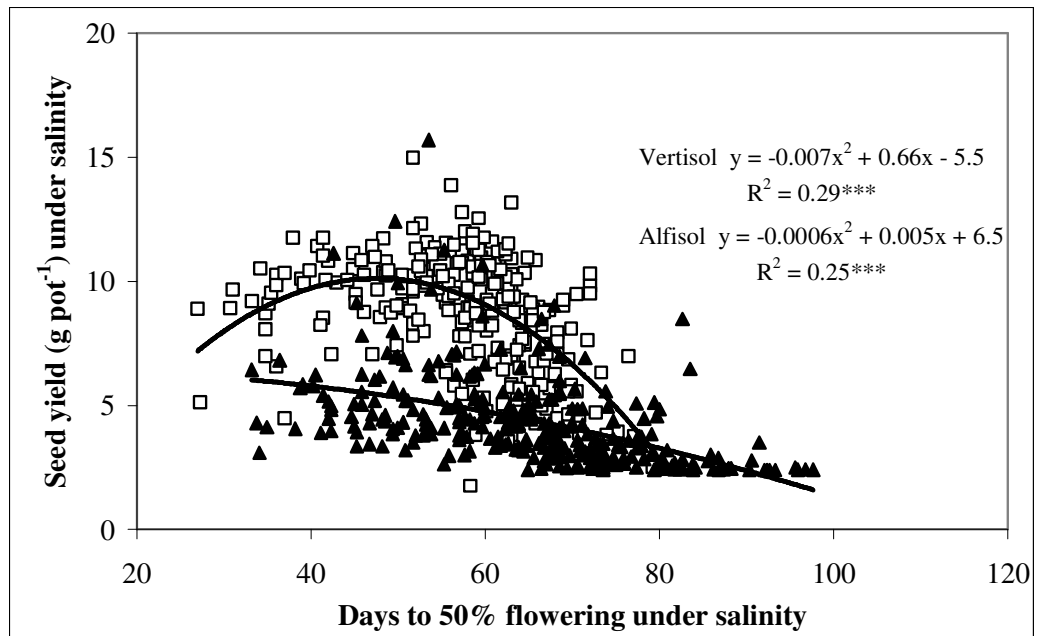
Accession	Vertisol			Alfisol		
	Days to 50% flowering	Shoot biomass (g pot <sup>-1</sup> )	Seed yield (g pot <sup>-1</sup> )	Days to 50% flowering	Shoot biomass (g pot <sup>-1</sup> )	Seed yield (g pot <sup>-1</sup> )
ICC 4814	45	30.6	9.5	65	20.0	3.4
ICC 15567	52	28.1	7.8	55	17.3	2.6
ICC 15697	58	32.4	7.8	73	21.4	2.9
ICC 13628	64	35.0	7.7	83	19.7	2.8
ICC 13441	72	38.7	7.6	79	22.2	3.0
ICC 6802	65	31.0	7.5	69	20.3	3.3
ICC 440	65	27.2	7.5	60	21.3	4.5
ICC 12037	64	31.9	7.4	71	21.8	3.1
ICC 3362	50	25.2	7.4	53	20.0	4.5
ICC 13764	61	36.3	7.3	71	24.1	4.2
ICC 3218	67	36.7	7.3	72	21.7	3.2
ICC 1194	58	31.9	7.1	63	20.4	3.5
ICC 12537	47	29.1	7.1	45	19.7	3.4
ICC 6537	63	27.0	7.0	64	23.3	5.4
ICC 12928	76	33.4	7.0	65	23.2	5.4
ICC 6571	61	31.1	6.9	70	21.8	4.1
ICC 6293	63	35.5	6.9	78	21.4	3.4
ICC 7323	62	34.2	6.9	73	19.4	2.5
ICC 6877	70	38.6	6.8	67	20.1	2.9
ICC 16487	67	33.0	6.8	74	21.9	2.8
ICC 8740	66	36.9	6.8	75	20.0	2.6
ICC 13187	61	41.4	6.7	76	24.1	3.6
ICC 7571	64	36.0	6.5	76	22.9	2.7
ICC 15406	62	36.4	6.4	61	22.2	3.3
ICC 16796	55	46.1	6.4	45	22.6	5.1
ICC 3421	66	37.9	6.3	72	21.1	2.6
ICC 7867	73	50.1	6.3	70	19.8	3.0
ICC 1923	68	36.1	6.3	57	20.2	3.7
ICC 7308	59	27.9	6.0	69	19.0	2.5
ICC 10341	65	41.7	5.9	66	25.5	5.2
ICC 2277	63	38.6	5.8	77	21.6	2.9
ICC 12492	66	34.6	5.8	76	22.1	3.3
ICC 11627	70	29.5	5.8	63	21.0	4.8
ICC 7315	56	30.7	5.8	62	23.9	4.5
ICC 9137	63	34.2	5.7	71	26.2	3.4
ICC 5135	68	32.1	5.7	67	22.3	4.3
ICC 12328	67	44.4	5.7	84	20.8	2.6

ICC 13523	64	32.9	5.7	64	21.5	3.3
ICC 2210	71	33.6	5.5	87	20.7	2.9
ICC 15510	65	31.5	5.5	67	22.0	5.2
ICC 10885	57	32.0	5.5	77	23.4	2.5
ICC 8261	60	34.6	5.3	64	23.5	3.2
ICC 15802	64	32.2	5.2	57	24.9	5.0
ICC 5504	62	36.9	5.1	69	25.0	5.5
ICC 13283	66	45.3	5.0	72	21.6	2.4
ICC 2065	67	28.8	4.9	73	20.2	2.7
ICC 6263	60	29.1	4.8	67	22.0	3.7
ICC 9402	70	51.4	4.7	82	20.0	2.4
ICC 12916	70	27.0	4.6	67	21.6	3.7
ICC 11764	71	46.1	4.6	74	24.7	3.5
ICC 16374	37	31.0	4.5	38	25.7	4.1
ICC 11584	68	28.6	4.5	73	20.5	2.6
ICC 15435	66	34.7	4.3	75	20.6	2.9
ICC 13357	64	49.0	4.1	84	22.2	2.5
ICC 2242	75	29.8	3.9	76	23.3	2.7
ICC 13077	71	48.3	3.9	75	23.6	3.4
ICC 8151	74	44.9	3.8	82	20.5	2.7
ICC 5337	63	41.0	3.8	90	20.8	2.4
ICC 15518	59	32.4	3.8	74	22.1	2.5
ICC 8522	77	40.6	3.8	93	20.5	2.4
ICC 10755	63	42.1	3.5	68	24.7	5.2
ICC 6306	79	40.9	2.5	96	24.8	2.4
ICC 1915	58	36.7	1.8	96	21.9	2.5

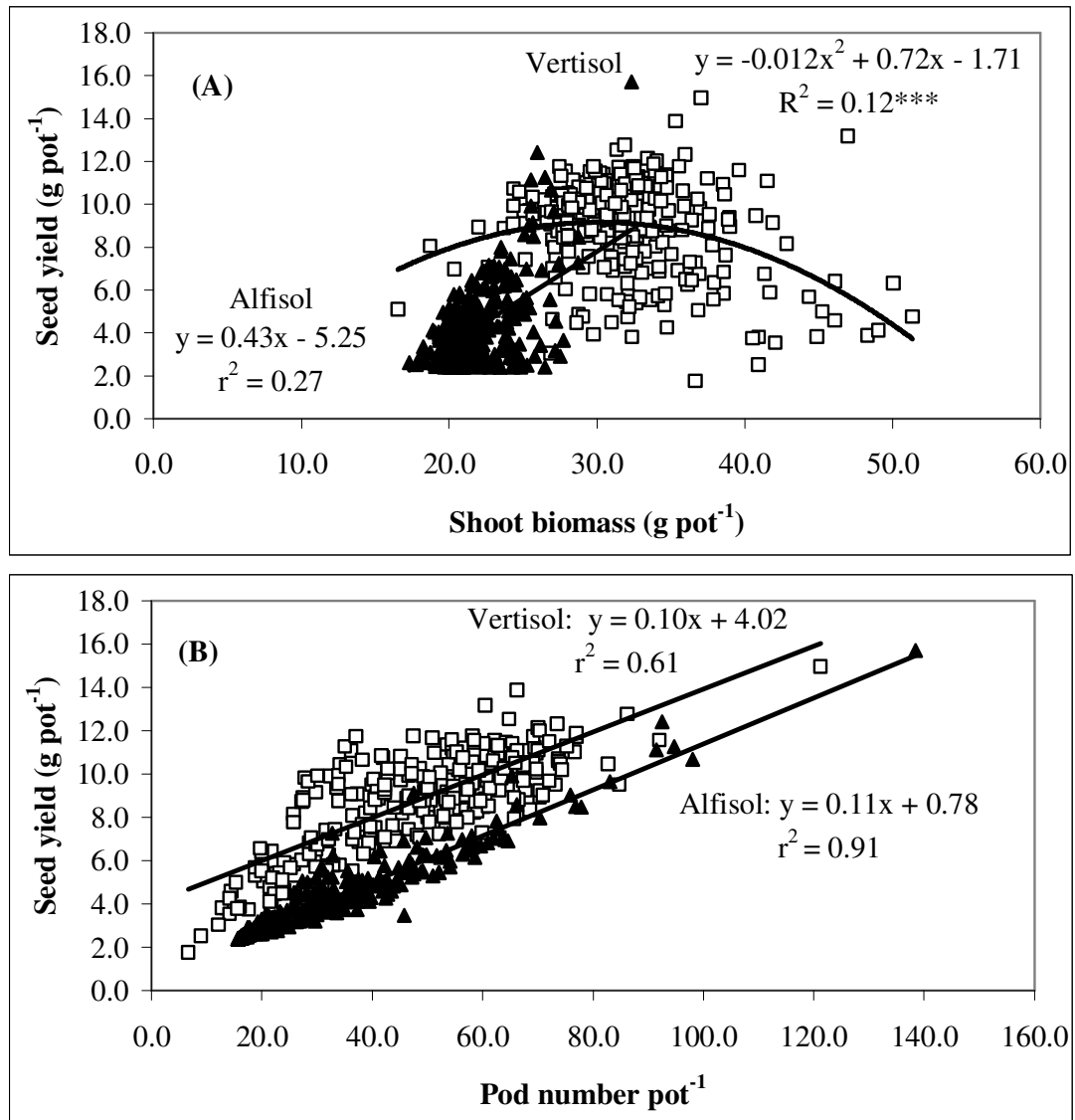
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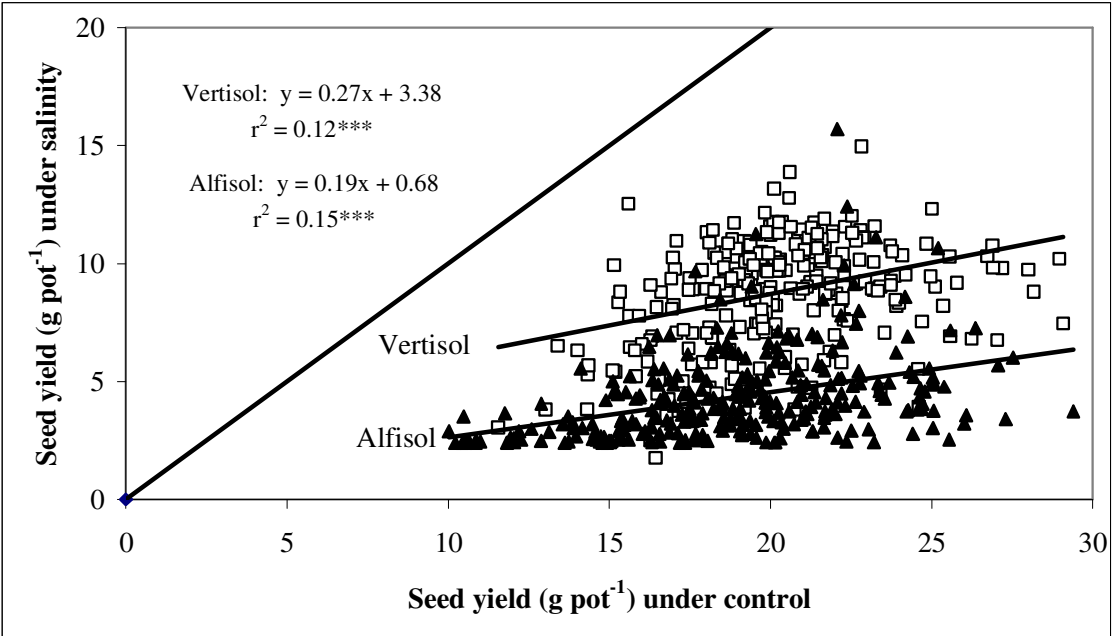
**Fig. 1** The relationship between 50% flowering (days after sowing) and the seed yield under salinity in a Vertisol soil in 2005-06 (open squares) and an Alfisol soil in 2007-08 (solid triangles).



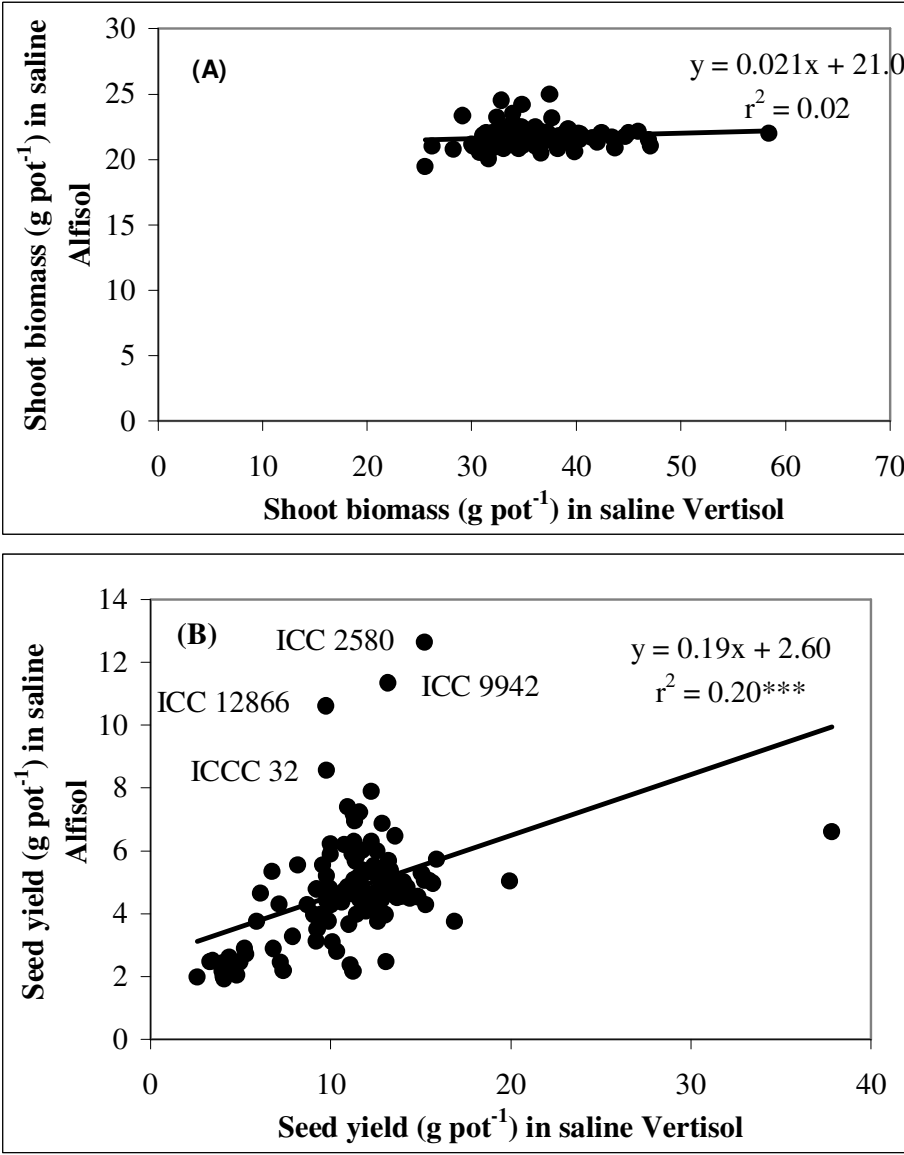
**Fig. 2** The relationship of seed yield of genotypes under salinity with that of (A) shoot biomass under salinity and (B) pod number under salinity in a Vertisol soil in 2005-06 (open squares) and an Alfisol soil in 2007-08 (solid triangles).



**Fig. 3** The relationship of seed yield in saline conditions with that of the seed yield in non-saline conditions in a Vertisol soil in 2005-06 (open squares) and an Alfisol soil in 2007-08 (solid triangles). The solid line is the 1:1 line



**Fig. 4** The relationship of (A) shoot biomass production in the saline Alfisol soil with that of the saline Vertisol soil, and (B) the seed yield in the saline Alfisol soil with that in the saline Vertisol soil.



**Fig. 5** The relationship between the pod numbers in the non-saline pots and in the saline pots in a Vertisol soil in 2005-06 (open squares) and an Alfisol soil in 2007-08 (solid triangles).

