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**Large number of flowers and tertiary branches, and higher reproductive success increase yields under salt stress in chickpea**

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## **A B S T R A C T**

Salinity is a major problem worldwide and improving salt tolerance of chickpea (*Cicer arietinum* L.) will allow expansion of production to more marginal areas. Plant reproduction suffers under salt stress in chickpea, but it remains unclear which process is most affected and what traits discriminate tolerant from sensitive lines. Three pot experiments were carried out to compare the effects of salt application (17 g NaCl kg<sup>-1</sup> Alfisol) at sowing (SS) and at the start of flowering (SF) on growth, canopy transpiration, plant architecture, and flower, pod and seed development (timing, numbers, mass, abortion). Six pairs of tolerant/sensitive lines with similar flowering times within each pair, but different among the pairs, were used. Shoot biomass was similar in tolerant and sensitive lines in the SS and SF treatments, whereas the seed yield decreased more under SS and SF treatments in the sensitive lines. The flower, pod and seed numbers within all pairs was higher in the tolerant than in the sensitive lines in the non-saline controls, but the differences in numbers of seeds and pods further increased in both the SS and SF treatments. By contrast, neither the duration of flowering or podding, nor the percentage of flower or pod abortion, discriminated tolerant from sensitive lines. In non-saline controls the numbers of primary branches was 100% higher across the sensitive lines, whereas the number of tertiary branches was 8-fold higher across tolerant lines. The relative transpiration of the tolerant lines in the salt treatments was above that for the sensitive lines in three pairs of tolerant/sensitive lines, but did not differ within two pairs. Our results demonstrate that constitutive traits, i.e. numbers of flowers and tertiary branches, and adaptive traits, i.e. high number of seeds under salt stress, are both critical aspects of salinity tolerance in chickpea.

**Key words:** Salinity stress, salt tolerance, reproductive biology, transpiration, flower abortion, pod abortion, *Cicer arietinum*

## **1. Introduction**

Salinity is a major and increasing problem worldwide that needs to be addressed in order to maintain agricultural production. Genetic approaches to improve crop tolerance of salinity (i.e. breeding) will be important, and especially since management options require a large investment that poor farmers are unlikely to implement. Chickpea is grown in various regions challenged by increasing soil salinity (Flowers et al., 2010). There exists genetic variation for salinity tolerance which can be used to breed superior varieties (Vadez et al., 2007; Krishnamurthy et al., 2011). However, breeding would be made more efficient by focusing on those traits that are critical, but still relatively unknown, for the salinity tolerance of chickpea (Flowers et al., 2010).

Although salinity affects shoot growth, its effect on reproductive processes is relatively more severe in chickpea. Genotypic tolerances, based on seed yield obtained under saline conditions, were related more to maintaining a large number of seeds and less to maintaining a high biomass production relative to a non-saline control (Dhingra et al., 1993; Vadez et al., 2007; Krishnamurthy et al., 2011). From the early development of flower meristems until the development of seeds in the pods, abiotic stresses can affect a number of processes. Abiotic stresses are known to affect meiosis during gamete production and male sterility appears to be more common than female gamete sterility (Saini, 1997). Flower production was decreased under drought in chickpea (Nayar et al., 2005; Fang et al., 2010), or under heat stress in groundnut (Prasad et al., 2000). Flower abortion was another cause for yield decrease under drought in a study that showed that cultivated chickpea aborted a larger number of flowers than wild germplasm (Nayar et al., 2005), or in chickpea exposed to cold where plants produced flowers but failed to set pods (Clarke et al., 2004; Srinivasan et al., 1998). Pod abortion also was the key limitation to seed yield in chickpea exposed to drought stress (Behboudian et al., 2001; Leport et al., 1999; 2006; Fang et al., 2010). Finally, the duration and rate of seed filling can also explain yield variations under drought stress in chickpea (Davies et al., 1999).

So, a number of processes during the reproductive phase can be affected by abiotic stresses. There is, unfortunately, limited knowledge on which of these processes are most affected in chickpea exposed to salinity and whether tolerant and sensitive lines differ in sensitivity of one or several of these processes to determine seed yield in saline soils.

Carbohydrate supply could be a limitation. Reproductive structures are quite demanding for carbohydrates and the supply of sucrose to the developing embryos was shown to be critical to rescue embryos of water stressed plants where photosynthesis was inhibited (Zinselmeier et al., 1999). So, reproductive failure under salt stress could be related to decreased transpiration relative to unstressed plants, where transpiration is a surrogate for photosynthesis/carbon supply to the developing embryos. Recent data (Vadez et al., 2011) also suggest that in early chickpea lines, where flowering is simultaneous with sustained shoot growth, the high yielding lines were those having both reproductive success and sustained shoot growth under saline stress. The sustained growth under saline stress could lead to increased branching and to an increased number of reproductive nodes and flowers. So, the question remains whether shoot growth and branching could lead to more reproductive structures, especially in early duration lines.

The overall objective of this work was to pinpoint traits that distinguish tolerant and sensitive lines, with a particular focus on plant architecture and reproductive biology. The work was performed with six pairs of tolerant/sensitive lines of chickpea in which flowering time was similar within each pair. A first objective was to compare effects of salt stress application at sowing and flowering on biomass and yield, with the hypothesis that salt effects would be the same in these two types of treatments if reproduction was the most sensitive plant process to salinity in chickpea. A second objective was to assess the effect of salt on phenological development (flowering/podding duration) and growth patterns (rooting, shoot branching). A third objective was to investigate the direct effects of salt treatment on reproductive structures (flower number and abortion, pod number and abortion, seed number and size). The last objective was to investigate how salinity affects plant transpiration during reproduction.

## **2. Material and methods**

## 2.1. Growth conditions and treatments

Chickpea (*Cicer arietinum* L.) was grown under saline and non-saline conditions in 20 cm diameter pots filled with 4 kg of Vertisol soil (Vertic Inceptisol) collected from the ICRISAT farm, mixed with farm manure at a rate of 50:1 (soil:manure, W/W), autoclaved, sieved and sun dried. The soil [pH 8.1, CEC/clay ratio = 0.8 and an electrical conductivity = 0.10 dS m<sup>-1</sup> in saturated paste extract (ECe) (El Swaify et al., 1985)], was fertilized with di-ammonium phosphate and muriate of potash, at a rate of 0.3 g and 0.2 g per kg soil, well mixed with the soil before filling the pots. Soil was inoculated with standard chickpea rhizobium inoculum at the time of sowing.

Three experiments were carried out between November and March at ICRISAT headquarters (Patancheru, AP, India, Latitude: 17° 31' 53 N, Longitude: 78° 15' 54 E), two outdoors (Experiment 1 and 2) and one in a greenhouse (Experiment 3). The average maximum temperatures ranged between 25.3 and 36.8 °C and minimum temperatures between 8.4 and 22.0 °C outdoors. The average maximum temperatures ranged between 29.7 and 32.6 °C and minimum temperatures between 15.4 and 16.1 °C in the greenhouse. Four seeds were planted in each pot. These were thinned to two plants per pot at three weeks after sowing.

Three treatments were used: A non-saline control (C), a salt treatment applied at the time of sowing (SS), and a salt treatment applied at the beginning of flowering (SF), therefore applied at different dates depending on genotype. The two salt treatments were equivalent and corresponded to a salt application in the irrigation water in sufficient quantity to wet the Vertisol to field capacity (1L per 4 kg pot) and result in the equivalent of 80 mM NaCl in the solution solution (1.17 g NaCl kg<sup>-1</sup> soil). The salt was applied in split applications. In SS, half the dose was applied at sowing by wetting the soil with 1 L of 40 mM NaCl solution, while the second dose was applied one week after sowing by adding 400 mL of 100 mM NaCl. In SF, half the dose was applied when all plants of a given pair of lines had started flowering, by flushing the pots with 1 L of 40 mM NaCl, and then the following day flushing again with 1 L of 80 mM NaCl. At each time, the non-saline control pots were also flushed with 1 L of water containing no salt. Lines ICC1431 and ICC6263 mistakenly received an additional L of 40 mM NaCl solution in the SF treatment, likely explaining their higher shoot, pod and seed mass decrease than

the other lines. Therefore, up to flowering time, the plants of the SF treatment and the C treatment were treated the same way. After salt application in the SS and SF treatments, 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, but also to avoid leaching of the salt.

## *2.2. Plant materials and details of experiments*

Experiment 1 (Exp.1) was carried out to compare the plant architecture, rooting, and timing of pod/seed production, the number of flowers and flower abortion, along with the seed yield/pod/seed number and shoot dry mass, and to assess the effect of salt stress on the rate of transpiration at the time of flowering (SF). Primary branches were those produced on the main stem, while secondary and tertiary branches were those produced on the primary and secondary branches, respectively. Exp.1 was conducted outdoors and used 5 pairs of lines that were classified as salt tolerant or salt sensitive based on seed yield in saline conditions in a previous evaluation (Krishnamurthy et al., 2011): ICC1431/ICC6263 (tolerant/sensitive in each case); JG11/ICCV2; ICC9942/ICC15802; ICC3512/ICC13283; ICC7819/ICC7571. These five pairs of lines had similar flowering time within pair, i.e. 37 DAS, 49-51 DAS, 49-51 DAS, 49 DAS, and 49 DAS, respectively. This was important because previous report showed higher tolerance to salinity in early duration lines (Vadez et al., 2007). Three treatments were used (C, SS, SF), each with 8 replicate pots per genotype. Four replicate pots per line and treatment were harvested at 30 days after treatment application in the SF treatment for assessing rooting and branching, whereas the other four replicates were kept until maturity.

Experiment 2 (Exp.2) was carried out to confirm the measurements of Exp.1, and contained an extra pair of tolerant/sensitive lines, with the objective to compare the yield reduction and flower/pod/seed number and abortion in the SS and SF treatments. In this experiment, no plants were grown in non-saline soil. Exp.2 was conducted outdoors and used 6 pairs of tolerant/sensitive lines (flowering time in parenthesis): ICC1431/ICC6263 (46-44 DAS); JG11/ICCV2 (35 DAS); ICC9942/ICC15802 (45-44 DAS);

ICC3512/ICC6877 (46 DAS); ICC7819/ICC7571 (46 DAS); ICC5845/ICC13283 (46 DAS). Four replicate pots per line were used in each of the two treatments (SS, SF).

Experiment 3 (Exp.3) was carried out to follow the duration of flowering and podding under salt stress, and to compare flower/pod/seed number and abortion in salt-treated/control plants. Exp.3 was conducted in a greenhouse and used three pairs of tolerant/sensitive lines (flowering time in parenthesis): ICC1431/ICC6263 (44 DAS); JG11/ICCV2 (34 DAS); ICC9942/ICC15802 (44 DAS). Three treatments were used (C, SS, SF), each with four replicate pots per line.

### *2.3. Flower and pod tagging*

In Exp.1, reproductive success was assessed in different weeks after salt application by tagging flowers appearing during an entire week with a thread of a particular colour, a different colour being used for each week so that at harvest flower production as well as pod and seed production could be followed on a weekly basis. In Exp.2 a similar principle was followed for flower tagging. In Exp.3, procedures followed closely Leport et al. (2006), and each flower was tagged at the time of appearance and the date written on the tag. Subsequently the date of pod appearance, if any, was recorded on the tag. Therefore the starts and ends of flowering and podding were documented for each plant, and for all three treatments (C, SS, SF). This tagging procedure also provided the total number of flowers produced by each plant (total number of tags per plant), the number of pods per plant (number of tags with a date of podding), the number of fertile pods (number of seeds per pod and plant, counted at harvest), and enabled calculation of the percentage abortion of flowers and pods.

### *2.4. Canopy transpiration*

Four days before salt application in the SF treatment of Exp.1, a uniform and thick layer of plastic beads was applied to the surface of each pot of each treatment. Previous work had shown that the beads reduced soil evaporation by about 90% so that weighing of pots could be used as a measure of plant transpiration (Ratnakumar et al., 2009). Eight replicate pots in each treatment and line were weighed every alternate day in the morning

from 4 days prior to until 4 weeks after the application of salt and the mass recorded. Transpiration rate was calculated from the pot mass differences and amounts of water added (pot mass day n minus pot mass day n+2 plus water added day n). Salt application was made at night and transpiration prior to salt application was made by weighing the pots late in the afternoon. After flushing the SF and C pots with their respective treatments, pots were let to drain overnight and pot masses taken again in the morning. This allowed the measurement of plant transpiration before and after the application of salt. Plants were then re-watered to 90% field (i.e. pot) capacity, to ensure that no water stress occurred and that there was no drainage.

Transpiration ratios (TR) were calculated for each day by dividing plant transpiration in SF by the mean of the transpiration in C for each line. Then normalized transpiration ratios (NTR) were calculated by dividing individual TR value by the mean of TR values before treatments were applied, for individual pots.

### *2.5. Harvest procedures*

When the plants reached maturity, primary, secondary and tertiary branches were counted. The two plants per pot were processed separately and the dates of flowering and podding on the tags and the number of tags per plant were recorded. Pods were separated, weighed after drying in a forced air oven at 70 °C for 2 days and counted. Pods were crushed and seeds were separated, weighed, and counted. Each plant was oven dried separately at 60 °C for 48 h and weighed for shoot dry mass.

In Exp.1, a set of plants (4 replicate pots in each of the C, SS and SF treatments) was also harvested at 30 days after SF treatment application to assess root biomass and branching patterns. At harvest in Exp.1, the ECe of the soil was measured in all pots following standard procedures (Krishnamurthy et al., 2011); soil ECe averaged 0.97 dS m<sup>-1</sup> in the SS treatment and 1.34 dS m<sup>-1</sup> in the SF treatment, therefore the salinity was slightly more severe in the SF treatment.

### *2.6. Statistical analyses*



The experimental designs were each a completely randomized block with treatment as the main factor and lines as the sub-factor; with eight replicates in Exp.1 and four replicates in Exp.2 and Exp.3. In Exp.1, eight replicates per line and treatment were used to assess the transpiration response to salt application in the SF treatment and compared to the C treatment. Then four of these replicates were used for the harvest at 30 days after SF application and the other four replicates were kept until maturity. One-way ANOVA was used for mean comparisons within treatments. Two-way ANOVA was used to compare C, SS and SF treatments and their interaction effects on the parameters measured.

### **3. Results**

#### *3.1. Comparison of salt application at different stages*

The shoot dry mass decreased by on average about 45% when salt was applied at the time of sowing (SS-Exp.1). However, lines varied in the degree of reduction in shoot dry mass from 25 to 80% (Table 1). By contrast, with salt applied at flowering (SF) the shoot mass was reduced on average by about 20%. There also, lines varied in the range of biomass reduction, with early duration lines JG11 and ICCV2 showing the largest decrease of 45 and 33%, respectively. In the C treatment, the average shoot dry mass of tolerant and sensitive lines were similar, and the reduction under SS was similar for tolerant and sensitive lines. Under SF, the shoot dry mass of the tolerant lines was reduced but not that of the sensitive lines (Table 1).

The pod mass was only slightly decreased in the SS treatment (about 13%). However, lines varied in the reduction of pod mass reduction. Sensitive lines had an average 24% decrease in pod mass under SS, whereas pod mass of tolerant lines did not differ from the control (Table 1). Results were very similar in the SF treatment: no significant decrease in pod mass overall, variation across lines in that percentage decrease, and an average decrease in the sensitive lines (21%), whereas the pod mass of the tolerant lines under SF was not different from the C treatment (Table 1). In addition, the mean pod mass of each group of lines was similar under SS and SF treatment. Nevertheless, the percentage changes for each line under SF and SS were poorly correlated, indicated also by a highly

significant genotype-by-treatment interaction (Table 1). This showed that some lines suffered the SS stress more than the SF stress (e.g. ICC13283), whereas others suffered the SF stress more than the SS stress (in particular early lines JG11 and ICCV2).

Results for the seed mass were very similar to those on pod mass for the SS treatment. The decrease in seed mass in the sensitive lines under both SS and SF treatments was about 25%, whereas the seed mass was unchanged compared to the C treatment in tolerant lines. The percentage changes also significantly varied for lines. For instance, the pod mass of tolerant JG11 decreased 15% under SF, whereas the seed mass increased 35%. Conversely, the pod mass of sensitive ICC13283 decreased 4% under SS, whereas the seed mass decreased 30% (Table 1). The two-way ANOVA revealed a predominant genotype (G) effect for pod and seed mass, although the genotype-by-treatment interaction was also highly significant.

In Exp.2 the results were similar to those from Exp.1, in particular the fact that tolerant and sensitive lines had on average similar seed mass under both SS and SF treatment. However, the average shoot dry mass of the tolerant and sensitive lines were similar under SS and SF. There also, the seed mass was about 60% higher in the tolerant (7.4 g per plant) than in the sensitive (4.3 g per plant) lines across both SS and SF treatment (Table 2). Also in both Exp.1 and Exp.2, the seed mass under SS within each pair of tolerant/sensitive lines was higher in the tolerant than in the sensitive lines, which confirmed earlier assessments on these lines (Vadez et al., 2007; Krishnamurthy et al., 2011). The two-way ANOVA revealed a major genotype effect on both shoot and seed mass, whereas the genotype-by-treatment interaction was not significant for seed mass (Table 2).

### *3.2. Phenological development*

The length of the flowering period, measured in Exp.3, was about 4 days longer in the tolerant than in the sensitive lines in the C treatment (33.0 versus 28.6 days) (Table 3). Under SS, this period was reduced by 17 and 15 days in the tolerant and sensitive lines. Under SF, the length of the flowering period was also significantly reduced compared to the C treatment by about 8 days in both the tolerant and the sensitive lines. The length of

the podding period was also slightly longer in the tolerant than in the sensitive lines in the C treatment (30.6 versus 26.0 days). Under SS, the length of podding was shortened by 16 and 15 days in the tolerant and sensitive lines. Under SF it was reduced by 9 and 8 days in the tolerant and sensitive lines. So, there appeared to be no major association between sensitivity to salt stress and the length of flowering or podding.

### *3.3. Plant morphology and development*

The numbers of primary branches were higher in the sensitive than in the tolerant lines under the non-saline C treatment, except in JG11/ICCV2 and ICC7819/ICC7571 (Table 4). The SS treatment significantly decreased the number of primary branches by 40% in the tolerant lines and 20% in the sensitive lines, whereas the SF treatment did not affect the number of primary branches. The number of secondary branches was similar in the tolerant and sensitive lines in the C and SS treatments. The SS treatment, but not the SF treatment, decreased secondary branching by 45% and 25% in the tolerant and sensitive lines. The trait of plant architecture that discriminated tolerant from sensitive lines the most was the number of tertiary branches under non-saline C conditions, which was more than 300% higher in the tolerant lines (22.1 per plant) than in the sensitive lines (6.2 per plant). The SS treatment decreased the number of tertiary branches dramatically, by 65% and 90% in the tolerant and sensitive lines, whereas the SF treatment significantly decreased the number of tertiary branches in the sensitive lines only (Table 4).

The root dry mass was higher in the sensitive lines than in the tolerant lines under non-saline C conditions (Table 4). The SS treatment dramatically reduced the root dry mass, by about 50% in both tolerant and sensitive lines so that the differences in root dry mass were no longer significant between tolerant and sensitive lines. Under the SF treatment, the root dry mass did not decrease in the tolerant lines, but significantly decreased in the sensitive lines so that the differences in root dry mass were no longer significant between tolerant and sensitive lines (Table 4).

### *3.4. Flower production and abortion*

The most remarkable difference was in the greater number of flowers produced under the non-saline C treatment in the tolerant lines (52.7 flowers) about 40% higher than in the sensitive lines (37.4 flowers). Under saline conditions the number of flowers was 75% higher in the tolerant than in sensitive lines in the SS treatment and 30% higher in the tolerant than in sensitive lines in the SF treatment (Table 5). In Exp.2, the number of flowers in the tolerant lines was about 100% higher than in the sensitive lines in both the SS and SF treatments (Table 6). Each of the six pairs of tolerant/sensitive lines tested showed a higher flower number in the tolerant than in the sensitive line, varying from 55% to 170% higher. Similar results were found in Exp.3 where the tolerant lines had a higher number of flowers (61.1 flowers per plant) than in the sensitive lines (27.8 flowers per plant) (Exp.3 – Table 3). Despite the variation in the total number of flowers within each pair of tolerant/sensitive lines, the trend was clear within each pair. Under SS and SF, the number of flowers of the tolerant also remained 75% and 55% higher than in the sensitive lines, with the trend being followed within each pair. Interestingly, ICC1431 and ICC6263 produced significantly more flowers under the SF than under the C treatment. So, a clear and confirmed trend across the experiments and lines was for a larger number of flowers in the tolerant lines, regardless of treatment, shown by a major genotypic effect on flower number, despite also a highly significant genotype-by-treatment interaction (Table 5 and 3).

In Exp.1 flower abortion in the C treatment was comparable in the tolerant and sensitive lines (70% and 79%) and similar results were found in the SS (83% versus 77%) and SF (88% versus 83%) treatments (Table 5). These results were confirmed in Exp.2 by similar flower abortion in the tolerant and sensitive lines in the SS (about 56%) and the SF (about 52%) treatments (Table 6). In Exp.3, the flower abortion was on average slightly higher (36%) in the tolerant than in the sensitive lines (20%) under C treatment. The lower flower abortion percentage in Exp.3 than in Exp.1 could be related to the fact that Exp.3 was carried out in the glasshouse under lower evaporative demand and possibly a milder effect of salt stress. The flower abortion decreased only slightly under the SS treatment (26% and 22%) and was similar in the tolerant and sensitive lines. This percentage remained relatively similar to the C treatment under SF (37% and 26%), and was not significantly different in the tolerant and sensitive lines (Table 3).

### *3.5. Pod and seed number, seed number per pod, and seed size*

In Exp.1, the number of pods was 28%, 43% and 89% higher in the tolerant than in the sensitive lines for the C, SS and SF treatments, respectively. The number of seeds was 41%, 125%, and 116% higher in the tolerant than in the sensitive lines for the C, SS and SF treatments, respectively (Table 5). Seed mass and seed number were closely related in Exp.1 ( $R^2 = 0.87$  for SS;  $R^2 = 0.77$  for SF). So, pod and seed number were generally higher in the tolerant than in the sensitive lines, regardless of treatment. Nevertheless, there was a highly significant genotype-by-treatment interaction effect, so that differences were larger between tolerant and sensitive lines under SS and SF conditions than in the C treatment. In Exp.1, the seed number per pod in the tolerant and sensitive lines was similar in the C and the SS treatment (0.87 versus 0.72), but higher in the tolerant than in the sensitive lines in the SF treatment (0.74 versus 0.51) (Table 5).

Results were confirmed in Exp.2 where the number of pods was 116% and 107% higher in the tolerant than in the sensitive lines for the SS and SF treatment respectively. Similarly, the number of seeds was 168% and 120% higher in the tolerant than in the sensitive lines for the SS and SF treatment, respectively (Table 6). The number of seeds per pod in Exp.2 was not significantly different in the tolerant and sensitive lines (0.98 versus 0.84 in the SS treatment; 1.03 versus 0.95 in the SF treatment) (Table 6).

In Exp.3 the number of pods was also 75%, 71% and 48% higher in the tolerant than in the sensitive lines for the C, SS, and SF treatment respectively. Similarly, the seed number was 69%, 82%, 45% higher in the tolerant than in the sensitive lines for the C, SS, and SF treatment, respectively (Table 3). The trend was similar within each pair of sensitive/tolerant lines. Pod abortion was similar in the tolerant and sensitive lines in the non-saline control treatment (22% and 21%), remained unchanged in the SS (20% and 17%) and in the SF (15% and 17%) treatments. Therefore, flower and pod abortion did not discriminate the tolerant from the sensitive lines (Table 3), but the number of seeds under salt treatment did.

The seed number was also followed week by week after flowering. Under all treatments, the number of seeds produced per plant per week decreased over time. Under

C conditions, the seed number per plant did not differ between the tolerant and sensitive lines in any of the four individual weeks following the initiation of flowering. By contrast, the seed number from week 1 and 2 was significantly higher in the tolerant than in the sensitive lines under both the SS and SF treatments (Fig. 1A). These results suggested that reproduction suffered the effect of salt application mostly in the two weeks following the beginning of flowering. The seed size of the sensitive lines was similar in the tolerant and the sensitive lines in all treatments.

### *3.6. Canopy transpiration*

The normalized transpiration ratio (NTR) remained above 1.0 in all tolerant lines, except at late stages after treatment in ICC3512 and ICC7819 (Fig. 2). The NTR was close to 1.0 in most sensitive lines and was even below 1.0 in ICC13283 (Fig. 2). In the first, second and third pair of tolerant/sensitive genotypes, the NTR of the tolerant was above the NTR of the sensitive lines, from about 10 days (Fig. 2A), 20 days (Fig. 2B), or 15 days (Fig. 2C) after treatment. In the fourth pair, the NTR was also occasionally higher in the tolerance than in the sensitive line (Fig. 2D). In the fifth pair, there was no significant difference in the NTR at any stage (Fig. 2E).

## **4. Discussion**

The treatment applied at flowering (SF) appeared to reduce the seed yield and related components by a similar magnitude to the treatment applied at the time of sowing. Nevertheless, there was some degree of interaction with the lines indicating that some were more sensitive to either one of times of imposing salinity stresses. Among the flowering characteristics, neither the duration of flowering and podding, nor the percentage of flower and pod abortion, discriminated the tolerant from the sensitive lines. Rather, it was the number of flowers produced under non-saline conditions that discriminated tolerant from sensitive lines in each of the pairs tested. Higher flower numbers resulted in higher numbers of pods and seeds per plant in the tolerant than in the sensitive lines under non-saline conditions. Under non-saline conditions, tolerant lines also had higher number of tertiary branches. In saline treatments (both SS and SF) the

differences between tolerant and sensitive lines in flower numbers and therefore pod and seed numbers were even greater. Seed number per pod was not different between the tolerant and sensitive lines in any treatment. The relative transpiration after treatment imposition was slightly higher in the tolerant than in the sensitive lines in three pairs of tolerant/sensitive lines.

#### *4.1. Timing of salinity stress: SF and SS treatment effects – genotype interaction*

The results showed that the effect of salt application from the time of flowering caused a similar effect on the yield components as the salt treatment from the time of sowing. By contrast, the shoot mass was more affected by the treatment applied from sowing than the treatment applied from the time of flowering. These results indicated that the effect of salt stress operated predominantly through an effect on reproduction, since well developed plants treated with salinity from flowering had similar yield reductions to those treated with salinity from the time of sowing, despite their lower shoot biomass (Table 1). This is in agreement with previous work that points to reproductive biology as the most salt-sensitive phase in chickpea (Dhingra et al., 1993; Katerji et al., 2005; Vadez et al., 2007; Krishnamurthy et al., 2011; Samineni et al., 2011). However, there was a significant genotype by treatment interaction. For instance early duration lines JG11 and ICCV2 also showed biomass reduction in the salt treatment applied at the time of flowering and so in the case of the early lines it is less clear whether a decrease in shoot biomass and/or a particular sensitivity of reproduction to salinity is the main cause for the decrease in seed yield. This agrees well with recent findings that in early-flowering chickpea lines, salinity tolerance is explained both by the maintenance of shoot growth and by the maintenance of a relatively large number of seeds, whereas in late-flowering lines it is only the latter trait, related to the success of reproductive biology, that is the basis of salt tolerance (Vadez et al., 2011).

#### *4.2. Constitutive traits that explain genotypic differences in salinity tolerance*

*Higher number of flowers* – Probably the most important finding of this work was that tolerant lines produced a much larger number of flowers than sensitive lines under non-

saline control conditions. This was confirmed in all three experiments, although with variation in the degrees of difference across experiments. Also important was the observation that the flower number did not decrease under the salt treatment applied at the time of flowering, neither outdoors (Exp.1), nor in the glasshouse (Exp.3), which suggest that salinity had no effect on the production of flowers in most lines. The flower number even increased under SF treatment in the tolerant line ICC3512 (Table 5). The flower number in the salt treatment applied at sowing did not decrease in the outdoor experiment (Exp.1), whereas it decreased in the greenhouse experiment (Exp.3) compared to the non-saline control, where the decrease in the flower number could have been a consequence of the limited growth in the SS treatment. The tagging procedure then allowed flowers to be followed through to maturity. The flower abortion percentage was similar in tolerant and sensitive lines across all treatments. So here, flower production was an important factor for seed yield under salinity, which differs from Leport et al. (2006) where pod abortion was the key factor for seed yield decrease under drought. The larger number of flowers was very closely related to the number of seeds under non-saline conditions ( $R^2 = 0.70$ ) and under saline conditions ( $R^2 = 0.55$ ).

*Tertiary branches* – Another striking result was the fact that plant architecture of tolerant lines differed from that of sensitive lines. Sensitive lines had a larger number of primary branches, but had much fewer tertiary branches than the tolerant lines. There was in fact a highly significant relationship between the number of flowers and the number of tertiary branches ( $R^2 = 0.34$ ), although the number of flowers showed a plateau once the number of tertiary branches was above 20. This is the first time that salinity tolerance in a crop has been reportedly related to plant architecture. As flowering nodes occur in leaf axils, a higher number of tertiary branches would expectedly relate to a larger number of flowers. Tolerant ICC9942, ICC1431, ICC 7819 had indeed large number of tertiary branches.

#### *4.3. Stress responsive traits that explain genotypic differences in salinity tolerance*

*Number of seeds per plant* – Tolerant lines had on average a larger number of seeds than the sensitive lines under control conditions and, as discussed above, this was related



to the larger number of flowers of tolerant lines under non-saline control conditions ( $R^2 = 0.70$ ). However, the differences in seed number between tolerant and sensitive lines were increased under both saline treatments in Exp.1. This was in part related to the even larger number of flowers in the tolerant than in the sensitive lines under salt stress ( $R^2 = 0.55$ ). Therefore, these results suggest that in addition to constitutive difference of sensitive lines producing less flowers, there are also differences in the number of seeds that tolerant and sensitive lines manage to retain under salt stress.

*Unrelated parameters (flower and pod abortion)* – Surprisingly, the larger number of seeds under salt stress in tolerant lines under both saline treatments was not related to differences in flower and pod abortion. The number of empty pods was also not significantly different between the tolerant and the sensitive lines. Seed number per pod under salt stress did not appear to be closely related to tolerance or sensitivity, although there was a tendency to have large number of seeds per pod in at least some of the tolerant lines under salt stress. The possibility that the flowering or the podding duration may be differentially affected in tolerant and sensitive lines was also considered. However, while the length of flowering was decreased in the salt stress applied at sowing, but not by the salt stress applied at flowering, and the length of podding was reduced in both saline treatments, none of these developmental periods showed large enough differences between tolerant and sensitive lines. However, the seed number per plant was clearly higher in the tolerant than in the sensitive lines, especially under SS and SF conditions. Our interpretation is that the number of empty pods, the percentage of pod abortion, the number of seeds per pod and small differences in flowering and podding times are components of the seed number per plant and may not have, individually, any significant effect, but have an additive effect, resulting in significant differences in the number of seed between tolerant and sensitive lines.

*Canopy transpiration and relation to seed fill* – Differences in the transpiration response to salt application were found in three pairs of tolerant/sensitive lines in which the relative transpiration of the tolerant line was above that of the sensitive line (Fig. 2). There were usually large experimental errors associated with such measurements. Nevertheless, the results indicated that tolerant lines were better able to maintain high assimilate rates (proxied here by canopy transpiration). An interesting fact was also the

higher root mass of sensitive lines under the non-saline treatment, but the lack of significant differences in the root mass of tolerant and sensitive lines under SS and SF treatment. Since salt stress induces an osmotic effect on plants (Munns and Tester, 2008), we may interpret that water absorption could have been root-limited under salt stress in the saline treatment in some sensitive lines. In agreement with that interpretation was the fact that the three sensitive lines having lower NTR than their respective tolerant counterparts were ICC6263, ICCV2, and ICC15802, and all had significant root dry mass reduction under SF treatment. These data would also agree with the previous hypothesis that saline stress reduces water absorption because of limited root growth (Whish et al., 2007).

## **5. Conclusion**

This work led to two major findings: (i) that constitutive traits largely affect the degree of tolerance of chickpea germplasm to salt stress (i.e. grain mass in saline conditions), although this trait discrimination is increased under saline conditions; (ii) that reproduction is indeed affected more by salinity in sensitive than in tolerant lines. The constitutive traits were the capacity of tolerant lines to produce a larger number of tertiary branches and a larger number of flowers, regardless of treatment. The stress-responsive traits were related to the capacity of tolerant materials to maintain a larger number of seeds under salt stress than sensitive lines, which we relate to a higher reproductive success, likely during the fertilization/development of the embryos. The present findings identify priorities for a number of mapping targets for these traits with three immediate possibilities: (i) number of tertiary branches; (ii) number of flowers; (iii) number of seeds per plant under saline conditions.

Supplementary material - Previous research showed that the relative decrease in seed yield was not related to the Na concentration in the shoot (Vadez et al., 2007). Here we found that the relative change in shoot Na concentration (shoot Na concentration in SS / shoot Na concentration in C) was negatively related to the relative seed yield (yield SS / yield C). However that relation was weak and depended on one data point with high

increase in Na concentration, and there was no relation in the SF treatment. By contrast, there was a highly significant correlation between the relative Na/K ratio (Na/K ratio SS / Na/K ratio C) and the relative seed yield ( $R^2 = 0.69$ ).

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## Figure Caption

**Fig. 1.** Seed number (per plant) (A) and 100-seed mass (g) (B) produced during weeks 1, 2, 3, and 4 after initiation of flowering in tolerant lines (T, closed symbols and solid lines) and sensitive lines (S, open symbols and dashed lines) exposed to non-saline control conditions (C, circles), saline conditions applied from the time of sowing (SS, triangles), and saline conditions applied from the time of flowering (SF, squares). Data are the mean data for each line within each of the tolerant/sensitive lines (n=5). Bars represent LSD (0.05%).

**Fig. 2.** Normalized transpiration ratio (NTR) in five pairs of tolerant/sensitive lines ICC1431/ICC6263 (A), JG11/ICCV2 (B), ICC9942/ICC15802 (C), ICC3512/ ICC13283 (D), ICC7819/ICC7571 (E) exposed to saline conditions from the time of flowering (SF). Tolerant lines are with closed symbols and solid lines. Sensitive lines are with open symbols and dashed lines. The arrows indicate the time of flowering and therefore the time of salt application. Data are means of 8 replicate pots. Bars represent LSD (0.05%).

**Supplementary Fig. 1.** Relationship between the percentage change in seed yield (i.e. seed yield under salt treatment/seed yield under non-saline treatment) and the percentage change in shoot Na concentration (Shoot Na concentration under salt treatment/Shoot Na concentration under non-saline treatment) (A), or the percentage change in the shoot Na/K ratio (Shoot Na/K ratio under salt treatment/Shoot Na/K ratio under non-saline treatment) (B). Data are the mean of 4 replicate pots per line and treatment (SF, open symbols, and SS, close symbols).

**Table 1**

Shoot (excluding pods), pod and seed mass (g per plant) at maturity in five pairs (Exp.1) of tolerant (T)/sensitive (S) lines exposed to non-saline control conditions (C), saline conditions applied from the time of sowing (SS), and saline conditions applied from the time of flowering (SF). Data are means of four replicate pots (x plants per pot, data expressed per plant). Two-way ANOVA (F-value) was used to compare line (G) treatment (T) and genotype-by-treatment effects (GxT). Least significant differences for each of the G, T, and GxT effects are provided.

	Shoot mass (g)			Pod mass (g)			Seed mass (g)		
	C	SS	SF	C	SS	SF	C	SS	SF
ICC 1431(T)	13.72	2.68	11.05	6.81	4.71	6.80	5.18	3.74	5.15
ICC 6263(S)	12.09	3.32	12.52	9.77	3.48	7.48	6.29	2.39	4.26
JG 11(T)	7.24	4.12	3.89	3.89	5.26	3.30	3.17	4.41	4.41
ICCV 2 (S)	6.56	2.60	4.40	4.44	3.12	2.11	3.07	2.35	2.35
ICC 9942(T)	10.49	6.63	11.18	10.05	9.54	7.08	8.14	7.64	5.33
ICC 15802(S)	12.84	7.88	12.20	9.61	7.58	6.16	6.84	5.58	4.16
ICC 3512 (T)	10.45	7.79	10.81	8.92	9.33	11.88	6.71	6.98	8.97
ICC 13283 (S)	14.51	12.89	18.59	7.74	3.22	7.40	5.48	2.00	3.91
ICC 7819 (T)	22.57	13.35	15.24	6.11	7.24	9.18	4.36	4.90	5.70
ICC 7571 (S)	17.19	10.49	13.77	5.03	7.77	5.67	3.72	5.41	3.52
LSD	4.60**	2.01**	3.01**	2.68**	2.50**	1.97**	2.11**	1.97**	2.03**
Mean tolerant	12.89	6.91	10.43	7.15	7.22	7.65	5.51	5.53	5.91
Mean sensitive	12.64	7.44	12.29	7.32	5.03	5.76	5.08	3.55	3.64
G effect	35.90**			20.31**			15.40**		
T effect	62.11**			4.75**			3.47*		
GxT effect	3.38**			5.19**			3.39**		
LSD <sub>G</sub>	1.87			1.31			1.10		
LSD <sub>T</sub>	1.02			0.71			0.60		
LSD <sub>GxT</sub>	3.24			2.26			1.91		

ns, non-significant, \* significant at  $P < 0.05$ ; \*\* significant at  $P < 0.01$



1 **Table 2**

2 Shoot, and seed mass (g plant<sup>-1</sup>) at maturity in six pairs (Exp.2) of tolerant (T)/sensitive  
 3 (S) lines exposed to saline conditions applied from the time of sowing (SS) and saline  
 4 conditions applied at the time from flowering (SF). Data are means of four replicate pots.  
 5 Two-way ANOVA was used to compare genotype (G) treatment (T) and genotype-by-  
 6 treatment effects (GxT). Least significant differences for each of the G, T, and GxT  
 7 effects are provided.

	Shoot mass		Seed mass	
	SS	SF	SS	SF
ICC 1431(T)	14.57	10.57	<b>9.14</b>	<b>7.56</b>
ICC 6263(S)	12.06	8.71	<b>6.04</b>	<b>3.67</b>
JG 11(T)	9.86	10.97	<b>7.72</b>	<b>9.73</b>
ICCV 2 (S)	5.89	4.84	<b>2.44</b>	<b>4.38</b>
ICC 9942(T)	15.79	11.76	<b>7.70</b>	<b>7.15</b>
ICC 15802(S)	12.06	15.60	<b>5.68</b>	<b>5.79</b>
ICC 3512 (T)	13.20	11.65	<b>6.66</b>	<b>7.16</b>
ICC 13283 (S)	15.57	15.73	<b>2.88</b>	<b>2.93</b>
ICC 7819 (T)	14.33	16.04	<b>8.83</b>	<b>6.08</b>
ICC 7571 (S)	18.86	15.47	<b>5.32</b>	<b>5.81</b>
ICC 5845 (T)	10.55	14.13	<b>6.22</b>	<b>4.54</b>
ICC 6877 (S)	17.15	20.86	<b>4.24</b>	<b>3.13</b>
LSD	5.30**	2.94**	1.68**	1.69**
Average tolerant	13.05	12.52	7.71	7.04
Average sensitive	13.60	13.54	4.43	4.28
G effect	9.94**		11.87**	
T effect	0.65 <sup>ns</sup>		1.56 <sup>ns</sup>	
GxT effect	1.97*		0.77 <sup>ns</sup>	
LSD <sub>G</sub>	3.11		2.26	
LSD <sub>T</sub>	ns		ns	

LSD <sub>GxT</sub>	4.40	ns
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8 ns, non-significant, \* significant at P<0.05; \*\* significant at P<0.01

**Table 3**

Length of the flowering and podding period (days), total number of flowers per plant, and percentage flower abortion, total number of pods, seeds, and empty pods per plant, percentage of pod abortion, in three pairs (Exp.3) of tolerant (T)/sensitive (S) lines exposed to non-saline control conditions (C), saline conditions applied from the time of sowing (SS) and from the time of flowering (SF). Data are means of five replicate pots. Two-way ANOVA was used to compare genotype (G) treatment (T) and genotype-by-treatment effects (GxT). Least significant differences for each of the G, T, and GxT effects are provided.

	Length of flowering			Length of podding			Number of flowers			Flower abortion percentage		
	C	SS	SF	C	SS	SF	C	SS	SF	C	SS	SF
ICC 1431(T)	22.5	15.1	25.1	19.1	12.1	22.7	51.7	20.5	67.2	32	31	35
ICC 6263(S)	30.6	16.3	27.9	26.5	14.4	25.0	36.0	14.9	52.7	28	32	36
JG 11(T)	40.8	17.3	28.4	36.9	14.6	21.5	58.2	21.0	37.3	40	26	39
ICCV 2 (S)	17.8	8.5	9.2	16.9	7.6	10.0	13.1	4.9	7.6	08	10	8
ICC 9942(T)	35.6	14.6	19.7	35.7	14.5	17.7	73.3	15.4	48.3	37	21	37
ICC 15802(S)	37.4	16.3	25.0	34.5	12.1	19.4	34.2	12.6	38.1	25	25	35
LSD	10.6**	5.2**	6.0**	11.2**	4.7*	8.5*	20.9**	4.0**	13.8**	14**	12**	12**
Average tolerant	33.0	15.7	24.4	30.6	13.7	20.6	61.1	19.0	50.9	36	27	37
Average sensitive	28.6	13.7	20.7	26.0	11.4	18.1	27.8	10.8	32.8	20	20	26
G effect	16.5**			7.86**			26.6**			15.3**		
T effect	61.7**			44.5**			74.3**			4.93**		
GxT effect	3.07**			2.72**			6.57**			1.23ns		
LSD <sub>G</sub>	4.1			4.74			7.6			0.07		
LSD <sub>T</sub>	2.89			3.35			5.4			0.05		

LSD <sub>GxT</sub>	7.1			8.21			13.2			Ns		
	Number of pods			Number of seeds			Number of empty pods			Pod abortion percentage		
	C	SS	SF	C	SS	SF	C	SS	SF	C	SS	SF
ICC 1431(T)	33.0	13.9	44.2	25.7	12.6	36.6	7.5	1.4	7.6	22	10	18
ICC 6263(S)	26.6	9.9	33.0	21.6	7.6	29.4	5.5	1.6	3.5	21	15	11
JG 11(T)	33.2	15.1	22.9	27.8	12.6	19.2	5.0	2.5	3.9	15	17	18
ICCV 2 (S)	12.0	4.4	7.0	10.9	3.8	6.0	1.5	0.6	1.0	17	14	12
ICC 9942(T)	46.0	12.1	31.4	32.4	10.3	26.6	13.0	1.9	4.9	31	19	23
ICC 15802(S)	25.2	9.7	26.3	18.2	8.2	21.3	6.8	1.5	5.2	25	23	28
LSD	13.0**	2.5**	11.0**	12.9*	2.5**	10.2**	5.7*	ns	2.9**	ns	ns	ns
Average tolerant	37.4	13.7	32.8	28.6	11.8	27.5	8.5	1.9	5.5	22	20	15
Average sensitive	21.3	8.0	22.1	16.9	6.5	18.9	4.6	1.2	3.2	21	17	17
G effect	19.4**			12.9**			7.12**			1.93ns		
T effect	61.0**			39.8**			26.2**			1.30ns		
GxT effect	4.51**			2.84**			2.95**			0.50ns		
LSD <sub>G</sub>	5.22			5.06			2.0			Ns		
LSD <sub>T</sub>	3.69			3.58			1.4			Ns		
LSD <sub>GxT</sub>	9.05			8.77			3.4			ns		

ns, non-significant, \* significant at P<0.05; \*\* significant at P<0.01

**Table 4**

Total number of primary, secondary and tertiary branches per plant and root dry mass (g per plant) in five pairs (Exp.1) of tolerant/sensitive lines exposed to non-saline control conditions (C), saline conditions applied from the time of sowing (SS), and saline conditions applied from the time of flowering (SF). Data are means of four replicate pots. Two-way ANOVA was used to compare treatment and treatment-by-genotype effect using only the SS and SF treatment. Two-way ANOVA was used to compare genotype (G) treatment (T) and genotype-by-treatment effects (GxT). Least significant differences for each of the G, T, and GxT effects are provided.

	Primary branches			Secondary branches			Tertiary branches			Root dry mass		
	C	SS	SF	C	SS	SF	C	SS	SF	C	SS	SF
ICC 1431(T)	5.7	2.0	5.5	32.0	4.3	23.2	31.0	0.7	35.2	4.6	0.7	4.2
ICC 6263(S)	19.2	12.2	15.0	43.2	3.2	25.7	11.7	0.0	2.5	5.5	1.0	3.6
JG 11(T)	11.5	12.0	15.0	17.0	14.2	24.0	4.5	0.2	0.5	2.4	1.3	1.9
ICCV 2 (S)	10.7	7.2	8.5	6.2	6.5	7.2	0.0	0.0	0.0	2.0	1.1	1.2
ICC 9942(T)	6.7	5.7	5.2	25.7	17.2	26.0	40.2	22.2	45.5	4.1	2.4	4.0
ICC 15802(S)	15.2	15.2	14.7	39.7	40.7	30.7	11.5	3.7	4.0	5.7	2.8	4.1
ICC 3512 (T)	16.0	6.0	17.0	35.0	19.5	33.7	12.7	8.7	12.2	4.4	3.7	3.8
ICC 13283 (S)	26.2	15.2	25.2	45.5	26.5	35.2	1.5	0.2	2.2	7.4	5.1	7.5
ICC 7819 (T)	16.2	6.5	12.2	62.2	34.7	37.2	28.7	12.0	26.5	7.0	4.1	6.5
ICC 7571 (S)	15.5	19.5	20.0	47.5	45.0	48.7	12.0	8.2	10.2	6.1	3.5	4.5
LSD	5.8**	4.6**	4.8**	10.5**	13.2**	7.6**	12.4**	6.1**	7.9**	1.2**	1.4**	1.4**
Average tolerant	10.0	6.4	10.7	27.4	13.8	26.7	22.1	8.0	23.4	3.9	2.0	3.5
Average sensitive	17.9	12.5	15.9	33.7	19.2	24.7	6.19	1.0	2.2	5.1	2.5	4.1

G effect	22.2**		21.1**		43.9**		23.9**				
T effect	22.4**		20.2**		57.8**		52.9**				
GxT effect	3.16**		3.12**		11.7**		2.97**				
LSD <sub>G</sub>	3.3		7.5		4.8		0.9				
LSD <sub>T</sub>	1.5		3.4		2.2		0.4				
LSD <sub>GxT</sub>	4.6		10.7		6.8		1.4				

ns, non-significant, \* significant at P<0.05; \*\* significant at P<0.01

**Table 5**

Number of flowers, percentage of flower abortion, number of pods and seeds per plant, seed number per pod, in five pairs (Exp.1) of tolerant/sensitive lines exposed to non-saline control conditions (C), saline conditions applied from the time of sowing (SS), and saline conditions applied from the time of flowering (SF). Data are means of four replicate pots. Two-way ANOVA was used to compare genotype (G) treatment (T) and genotype-by-treatment effects (GxT). Least significant differences for each of the G, T, and GxT effects are provided.

	Number of flowers			Flower abortion			Number of pods			Number of seeds			Seed number pod <sup>-1</sup>		
	C	SS	SF	C	SS	SF	C	SS	SF	C	SS	SF	C	SS	SF
ICC 1431(T)	52.0	35.0	51.8	76	76	83	39.7	27.0	43.0	33.3	30.7	36.0	0.86	1.16	0.84
ICC 6263(S)	45.8	18.5	40.5	77	72	91	35.5	13.8	37.0	25.5	10.5	20.0	0.72	0.79	0.55
JG 11(T)	26.8	27.8	25.3	68	88	86	18.3	24.5	22.0	14.8	22.5	14.0	0.83	0.92	0.63
ICCV 2 (S)	26.3	14.3	14.0	86	97	91	22.0	13.8	12.8	16.5	11.8	8.0	0.73	0.95	0.63
ICC 9942(T)	70.8	66.5	52.5	73	88	86	51.3	58.8	45.0	62.8	65.3	40.8	1.23	1.12	0.92
ICC 15802(S)	51.3	42.0	47.0	78	86	86	40.0	35.8	40.5	34.0	24.8	19.0	0.85	0.71	0.48
ICC 3512 (T)	61.3	70.5	80.8	78	91	93	48.0	64.0	75.3	36.3	42.5	61.3	0.76	0.67	0.81
ICC 13283 (S)	33.0	29.0	55.7	80	59	70	26.3	18.3	39.3	22.0	10.5	17.7	0.84	0.53	0.46
ICC 7819 (T)	53.0	71.3	64.3	56	71	90	29.5	50.5	57.5	17.0	23.3	28.0	0.58	0.47	0.49
ICC 7571 (S)	30.7	51.5	52.5	72	73	75	22.0	37.5	40.0	18.0	24.3	18.5	0.81	0.64	0.45
LSD	8.9**	4.6**	3.3**	15*	14**	10**	10.8**	12.5**	11.3**	9.3**	11.5**	9.9**	0.22**	0.29**	0.19**
Average tolerant	52.7	54.2	54.9	70	83	88	37.33	44.95	48.5	32.82	36.8	36.0	0.85	0.87	0.74
Average sensitive	37.4	31.0	41.9	79	77	83	29.17	23.80	33.9	23.20	16.3	16.6	0.79	0.72	0.51

G effect	13.9**		7.18**		40.0**		52.8**		12.9**						
T effect	19.9**		16.87**		11.3**		0.70ns		15.9**						
GxT effect	3.90**		2.88*		5.4**		5.57**		1.9*						
LSD <sub>G</sub>	3.3		0.07		6.4		5.7		0.13						
LSD <sub>T</sub>	1.8		0.04		3.5		ns		0.07						
LSD <sub>GxT</sub>	5.7		0.12		11.1		9.8		0.23						

ns, non-significant, \* significant at P<0.05; \*\* significant at P<0.01



**Table 6**

Number of flowers, pods and seeds per plant, seed number per pod, and percentage of flower abortion in six pairs (Exp.2) of tolerant (T)/sensitive (S) lines exposed to saline conditions applied from the time of sowing (SS) and saline conditions applied from the time of flowering (SF). Data are means of four replicate pots. Two-way ANOVA was used to compare genotype (G) treatment (T) and genotype-by-treatment effects (GxT). Least significant differences for each of the G, T, and GxT effects are provided.

	Number of flowers		Flower abortion percentage		Number of pods		Number of seeds		Seed number per pod	
	SS	SF	SS	SF	SS	SF	SS	SF	SS	SF
ICC 1431(T)	117.7	125.1	41	51	68.4	58.3	76.0	63.3	1.11	1.10
ICC 6263(S)	77.5	80.1	50	60	38.0	31.7	26.9	29.4	0.71	0.93
JG 11(T)	106.6	125.9	48	53	48.0	54.1	42.1	48.9	0.87	0.91
ICCV 2 (S)	28.4	55.0	16	52	19.0	25.4	16.4	24.3	0.88	0.99
ICC 9942(T)	110.6	97.4	36	32	70.4	64.9	75.2	54.6	1.09	0.87
ICC 15802(S)	73.4	54.7	57	51	31.0	25.9	25.7	23.3	0.83	0.91
ICC 3512 (T)	154.0	124.1	64	63	52.6	46.1	48.6	44.4	0.92	0.98
ICC 13283 (S)	54.4	45.7	71	63	16.6	17.0	13.9	17.3	0.91	1.12
ICC 7819 (T)	129.7	96.0	60	65	52.2	33.1	41.7	38.3	0.79	1.25
ICC 7571 (S)	67.0	54.7	56	43	30.3	27.3	24.4	25.0	0.84	0.92
ICC 5845 (T)	188.0	160.1	66	72	58.4	45.6	64.3	46.3	1.10	1.04
ICC 6877 (S)	85.9	69.6	69	71	27.0	18.4	23.0	15.0	0.83	0.82
LSD	25.6**	23.6**	24**	16**	12.2**	12.0**	12.0**	11.4**	0.15**	ns
Average tolerant	134.4	121.4	52	56	58.3	50.4	58.0	49.3	0.98	1.03

Average sensitive	64.4	60.0	53	57	27.0	24.3	21.6	22.4	0.84	0.95
G effect	18.2**		3.70		20.89**		24.98**		2.99**	
T effect	0.99ns		0.10ns		7.34**		4.86*		0.64ns	
GxT effect	1.00ns		0.75ns		0.86ns		0.60ns		0.30ns	
LSD <sub>G</sub>	26.3		0.18		14.1		14.5		0.21	
LSD <sub>T</sub>	-		-		-		-		-	
LSD <sub>GxT</sub>	ns		ns		ns		ns		ns	

ns, non-significant, \* significant at P<0.05; \*\* significant at P<0.01

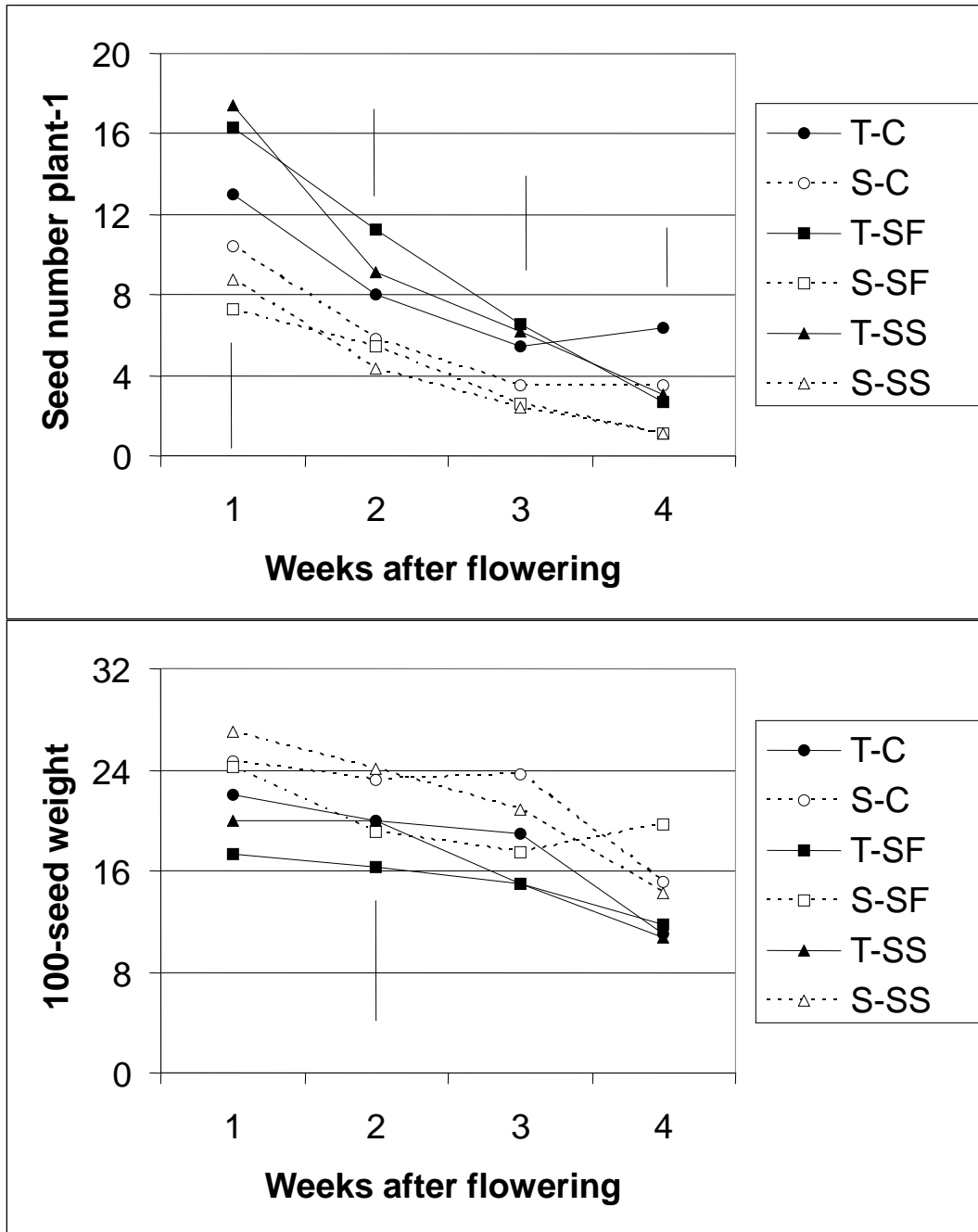
### **Highlights:**

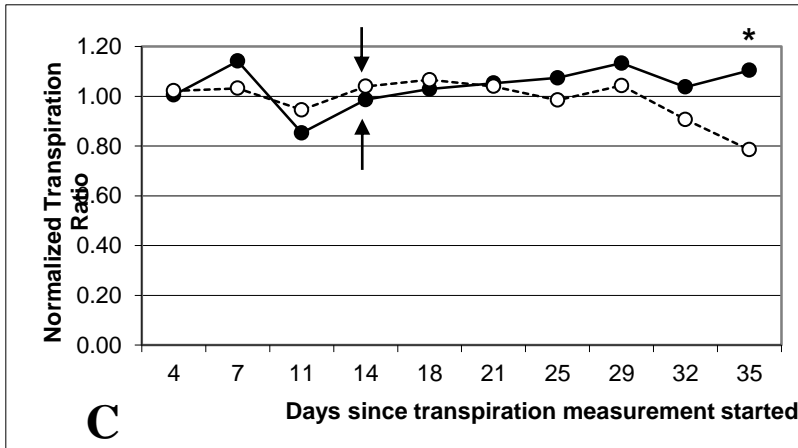
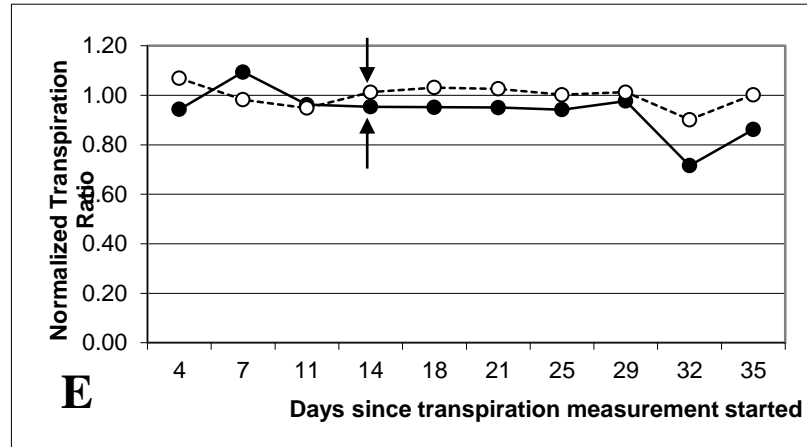
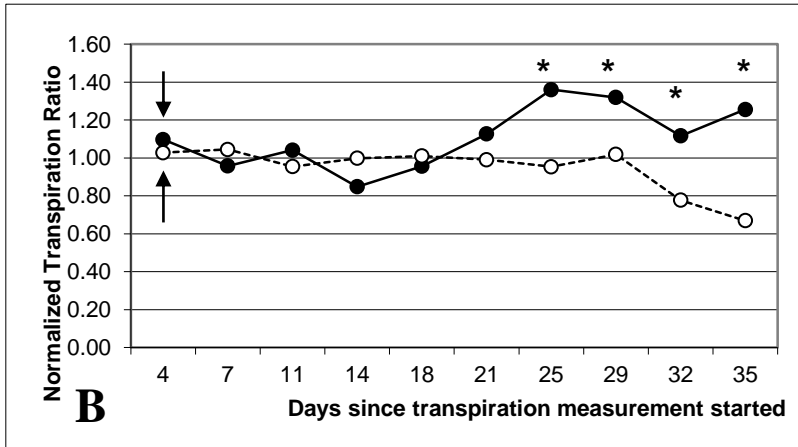
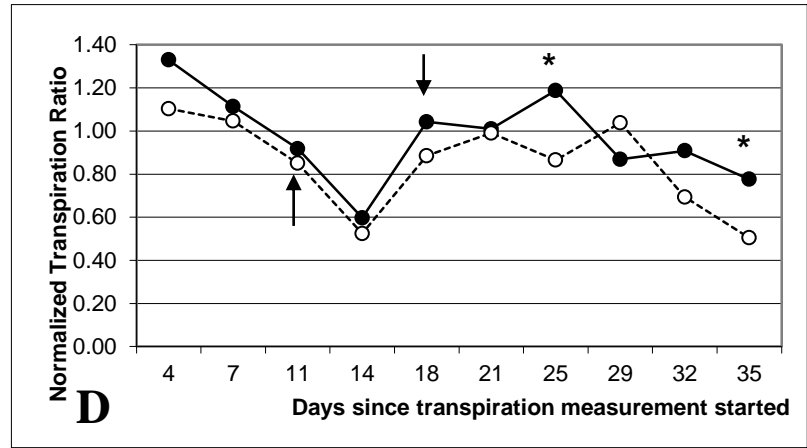
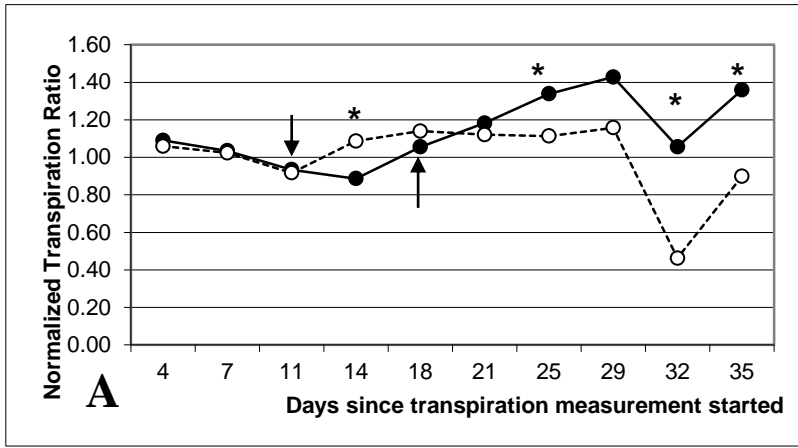
Salinity tolerant lines have larger number of flowers and tertiary branches under non-saline conditions than sensitive lines.

Salinity tolerant lines maintain a larger number of seeds under saline conditions than sensitive lines, which probably relates to the higher reproductive success under salt stress of tolerant lines.

Both constitutive traits (larger number of flower and tertiary branches) and adaptive traits (higher reproductive success under salinity) are key factors for chickpea tolerance to salinity - This clarifies breeding target and opens interesting future research.

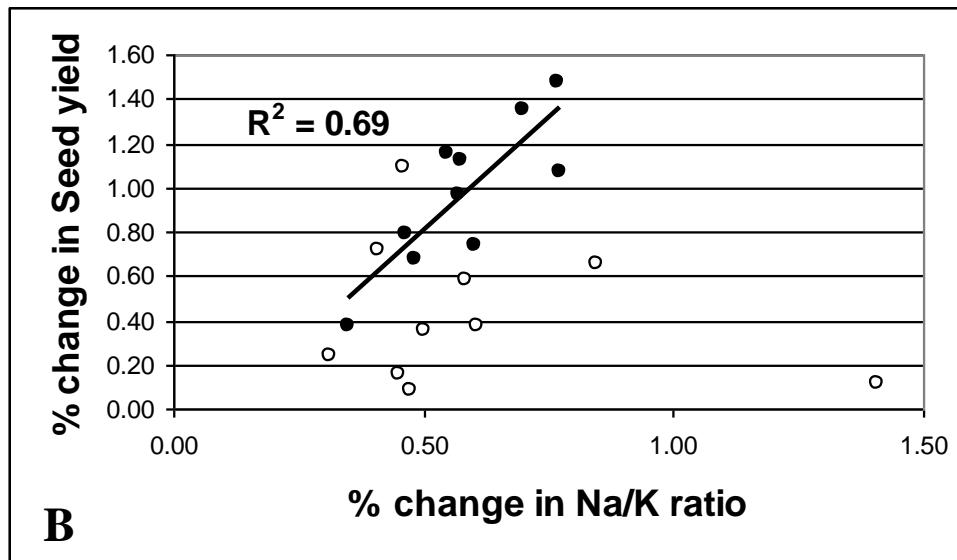
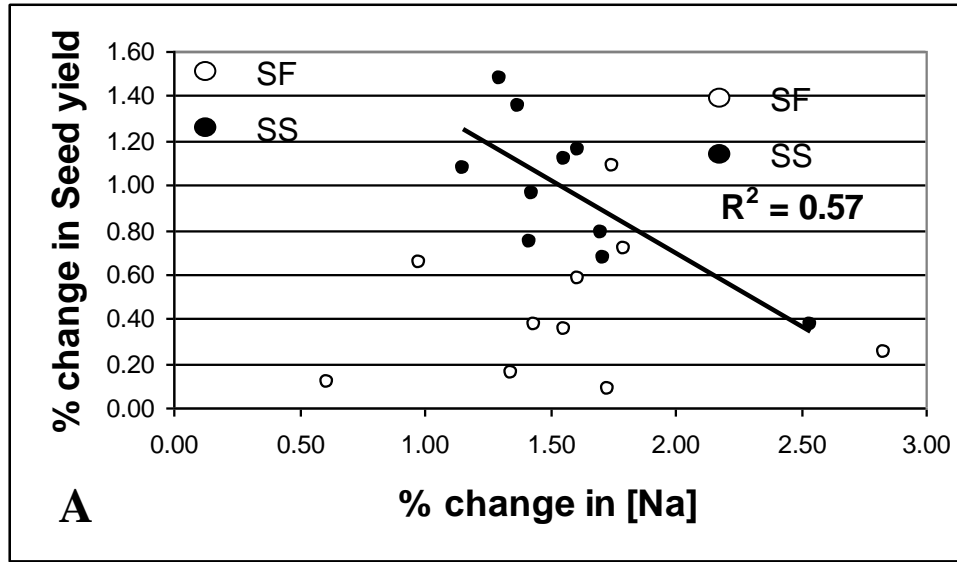
Figure 1





● Tolerant  
○ Sensitive

Figure 2



Supplementary Figure 1