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Assessment of ICCV 2 × JG 62 chickpea progenies shows sensitivity of reproduction to salt stress and reveals QTLs for seed yield and yield components

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Abstract

Salinity is a complex abiotic stress and understanding the physiological and genetic basis of salinity tolerance is a prerequisite for improving existing crop cultivars. Experiments were undertaken using 126 recombinant inbred lines from a cross between JG 62 (tolerant) and ICCV 2 (sensitive) to characterize traits related to seed yield differences under saline conditions and to map quantitative trait loci (QTL). The population segregated for flowering time and entries were separated into ‘early’ and ‘late’ phenology groups to undertake the analysis. In both groups seed yield varied under salinity, with

seed number being the most closely related trait to yield. In contrast, seed yield was not related to 100-seed weight or flowering time. Shoot dry weight was positively correlated with seed yield in the early entries only, but had no significant relationship with seed number. The higher sensitivity to salinity of the early entries was related both to a smaller biomass and lesser seed number under saline conditions. A QTL for seed yield under saline conditions was found in linkage group 3 in the late group, and a cluster of QTLs for seed yield components in linkage group 6, including a QTL for seed number which explained 37% of the variation. In contrast, no QTL for seed yield was found in the early group, but a QTL for seed number under saline conditions was found. These data indicate that salinity tolerance traits are linked to the degree of earliness in chickpea. Tolerance is determined by the success of reproductive sites in both early and late entries, which relates in part to constitutive traits, and by the capacity of maintaining growth in early-flowering lines only. This is the first report of QTLs for seed yield and seed number in chickpea exposed to salinity.

Key words Salinity . Chickpea . Recombinant Inbred Lines . Quantitative Trait Loci . Yield . Seed Number . Days to flowering .

Introduction

Salt stress is one of the major abiotic stresses—ranking only second to drought—which affects crop productivity in many parts of the world (Rangasamy 2006). Salinity continues to increase due to mobilization of salts to the root zone (secondary salinity) owing to changes in the pattern of vegetation cover in many regions. There are increasing numbers of cases where salinity occurs from mismanaged irrigation practices, especially in areas where evaporation is high. Thus, salinity is an increasing threat for agriculture in many regions.

Chickpea is sensitive to salinity (Lauter and Munns 1986; reviewed by Flowers et al. 2010) and field salinization in part explains the displacement of chickpea production from north India to south India. Recent reports, however, show large variation in seed

yield among a large, representative set of chickpea genotypes (Vadez et al. 2007; Krishnamurthy et al. 2011). Despite the relative sensitivity of chickpea to salt stress, tolerant and sensitive lines exist that can be used to better understand tolerance mechanisms and assist in breeding lines with improved tolerance (Munns and Tester 2008). In previous research, lines ICCV 2 and JG 62, parents of an existing mapping population developed for double poddedness in chickpea (Cho et al. 2002), were reported as sensitive and tolerant (low and high seed yield under salinity), respectively (Vadez et al. 2007). This provided an opportunity to identify traits related to differences in tolerance and to map quantitative trait loci (QTLs) for such traits, within this population.

Seed yield under salinity, measured in a short season environment, was related to flowering time in chickpea and followed an inverted parabola, with an optimum about 55 days after sowing (Vadez et al. 2007). Both early and late maturing genotypes yielded less well, whereas mid-duration lines tended to have the highest yields under saline stress. Since ICCV 2 flowers early (about 30–35 days after sowing), about 10 days earlier than JG 62, their phenological differences explain in part their yield differences under saline conditions. Therefore, an important question is addressed here about the segregation for seed yield under salinity in ICCV 2 × JG 62 recombinant inbred lines (RIL) progenies and its relation to their segregation for flowering time. A second question is whether QTLs for seed yield and putatively related traits can be identified within or across ‘early’ and ‘late’ groups for flowering time. Two years of testing are reported, in which different severities of salt stress were imposed in an outdoor artificially-salinized soil pot system, enabling discrimination for salt tolerance amongst the RILs.

Although many studies have evaluated salinity tolerance in chickpea on the basis of biomass differences at vegetative stages (see Flowers et al. 2010), recent work has clearly shown that salinity tolerance is not related to the capacity of genotypes to maintain biomass production or to fill seeds (seed size) under salt stress (Vadez et al. 2007). Rather, tolerance was related to the capacity of genotypes to maintain a large number of seeds (i.e. filled pods), indicating that salt tolerance in chickpea is related to tolerance of reproductive sites (Mamo et al. 1996; Katerji et al. 2001; Samineni et al. 2011). Such relationships and mechanisms have not been tested in early maturing chickpea lines; such

research is needed since chickpea production is expanding in short cropping season environments (<http://test1.icrisat.org/ChickPea/Chickpea.htm>).

The overall objective of this work was to map QTLs for salinity tolerance, using 126 RILs from a cross between salt sensitive ICCV 2 and salt tolerant JG 62. The specific objectives were: (i) to evaluate the interdependence of salt tolerance and flowering time; (ii) to test the relationship between seed yield under saline and non-saline conditions; (iii) to test the relationship between seed yield and its components (shoot biomass, seed and pod numbers, 100-seed weight) under salt stress; and (iv) to identify QTLs for seed yield and components, within and across two maturity groups.

Materials and Methods

Plant growth and treatment applications

Two experiments were carried out in two different growing seasons, 2005–06 and 2007–08. Plants were grown under saline and non-saline conditions in 27 cm diameter pots containing 7.5 kg of vertisol soil from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) farm, as previously reported (Vadez et al. 2007). The soil was fertilized with diammonium phosphate and muriate of potash, both at a rate of 300 mg kg⁻¹ soil. The experiments were carried out between November and February (planted on 22 Nov 2005 and 3 Nov 2007) at ICRISAT headquarters (Patancheru, AP, India) in an open-air facility equipped with portable rainout shelters to prevent interference from possible rain. The average maximum and minimum temperatures were 29.4 and 12°C, respectively, in 2005–06, and 29.8 and 13.9°C in 2007–08.

In 2005–06, the saline treatment had 8.77 g NaCl pot⁻¹ (equivalent to 1.17 g NaCl kg⁻¹ soil) applied at sowing as 80 mM NaCl solution in a sufficient volume to wet the soil to field capacity (1.875 L pot⁻¹ = 25% w/w). In 2007–08, salt application was increased to 10.96 g NaCl pot⁻¹ (equivalent to 1.46 g NaCl kg⁻¹ soil) to increase the discrimination between entries. In 2007–08, the treatment was applied in two half-doses (equivalent to 5.48 g NaCl pot⁻¹ each time), as 1.875 L of a 50 mM NaCl solution at sowing and 1.0 L of a 94 mM NaCl two weeks after sowing, which together is equivalent to a 1.875 L of a

100 mM NaCl solution. Thereafter, pots were watered with tap water containing no significant amount of NaCl. The bottoms of the salinity-treated pots were sealed to avoid any salt leakage, while those of the non-saline controls contained drainage holes. Utmost care was taken to avoid over-watering the salinity-treated pots, whilst maintaining pots close to field capacity to avoid any increase in salt concentration. This was achieved by applying a set amount of water to all pots, and this amount was set at each re-watering to the amount of water needed by the smallest plants in the trial. Thereafter, the largest plants in the trial received additional water to replace that used, based on the dryness of soil in these particular pots and on experience from several years running such large scale experiments in this soil (e.g. Vadez et al. 2007; Krishnamurthy et al. 2011). Non-saline treated controls were maintained close to field capacity by regular watering. In both treatments, six seeds of a single RIL were planted in each pot and all pots were later thinned to four plants per pot. The experiment was a randomized block design with two treatments (saline and non-saline) and four replicated pots for each entry within each treatment.

Plant material

The experiments were carried out on 126 F₁₂ RILs from the cross between ICCV 2 and JG 62, along with the parental lines. Genotype ICCV 2 is an extra-early line which usually flowers in less than 30–35 days, while JG 62 is a variety with two pods per node that flowers about 10 days later. The RIL population was previously developed to identify genes/QTLs related to the double podding trait (Cho et al. 2002). ICCV 2 was identified as being significantly more salt-sensitive than JG 62 (low and high seed yield under saline conditions, respectively) by Vadez et al. (2007).

Traits measured

Time to 50% flowering (i.e. at least 2 of 4 plants flowering) was recorded on each pot. Plants were harvested at maturity and the following measurements recorded: time to maturity (when 50% of the plants in each pot were fully mature), shoot biomass (g pot⁻¹),

pod weight (g pot⁻¹), seed weight (g pot⁻¹), seed number per plant, pod number per plant and 100-seed weight. Shoot, pod and seed samples were oven-dried at 70°C for 2 days.

Marker genotyping and linkage map construction

Genotyping data were generated or compiled for 216 markers in a separate study (Anuradha et al. 2011). The marker genotyping data were analyzed using χ^2 -test to test the goodness-of-fit to the expected 1:1 segregation ratio for each marker. Subsequently, the genotyping data of all markers, including those that showed segregation distortion, were used to construct a linkage map at LOD threshold grouping values of 15 using MAPMAKER (Lander et al. 1987) and Kosambi mapping function (Kosambi et al. 1944). As the map distance was unusually large at lower LOD thresholds, higher LOD thresholds were chosen to eliminate spurious linkage among markers.

QTL identification

Composite interval mapping (CIM) with 1000 permutations was done using QTL Cartographer (Wang et al. 2010). QTL identification was done for the two phenology groups (early and late) separately and together. When analyzing an individual phenology group, the other group RIL data was considered missing. The analysis was also done within and across both years.

Statistical analysis

A two-way ANOVA was carried out within each group of entries to assess the affect of salt treatment and of the genotype-by-treatment interaction. Then a one-way ANOVA was carried out to assess the genotype effect for the different traits measured within each treatment, year of experiment, and group of phenology (early and late – see below). Unbiased estimates of variance components σ^2_g and σ^2_e , were calculated, from which heritability was estimated as $h^2 = \sigma^2_g / (\sigma^2_g + \sigma^2_e)$.

Results

Population segregation for flowering time and effect of salt stress

The RIL population is known to segregate for flowering time under non-saline conditions; the first objective was to assess the segregation for flowering time in the mapping population under salinity, prior to considering yield responses to salinity. Since there was a close agreement between flowering time across years in a given treatment ($R^2 = 0.81$ and 0.77 under saline and non-saline conditions), flowering times were averaged for each genotype within a treatment across years. The frequency distribution of flowering time under non-saline control conditions identified an 'early' and 'late' group with flowering times ranging from 29 to 40 days after sowing (DAS) and from 42 to 54 DAS, respectively. Similarly, under saline conditions, entries segregated into an 'early' and a 'late' group with flowering times ranging from 29 to 38 days and from 41 to 56 days after sowing, respectively (S1 - Supplementary figure).

Except for eight entries from the 'early' flowering group under non-saline conditions that were somewhat delayed under saline conditions, flowering times across treatments were closely related (S2 - Supplementary figure). Therefore, given the previous report of an interaction between yield under salinity and time to flowering in chickpea (Vadez et al. 2007), further analysis of yield and component responses to salinity was then conducted considering separately the two phenological groups identified in the saline treatment, i.e. 29 to 38 DAS (early) and 41 to 56 DAS (late) (S1 - Supplementary figure).

The slope of the regression equation between flowering time under non-saline and saline conditions indicated that as flowering time increased, the delay in flowering under salinity increased (regression equation above the 1:1 line, S2 - Supplementary figure). This resulted in a significant delay as a result of salinity in mean flowering time in the early group of 3 days in 2005–06 and -1 days in 2007–08, while the mean delay in flowering time of the late group was 5 days in 2005–06 and 4 days in 2007–08 (Table 1), a delay that was also significant.

Effect of salt stress on seed yield and components in early and late phenology groups

Salt treatment had a significant effect on days to flowering, seed yield, shoot dry weight, pod number, seed number and 100-seed weight in both groups in both years, except on seed yield in 2005-06 in the late group. In 2005-06, the genotype-by-treatment interaction was significant for all parameters, except for the 100-seed weight in the early group, although the magnitude of the interaction was somewhat lower in the late group. In 2007-08, the genotype-by-treatment interaction was significant for all parameters except pod number in the early group. By contrast, the genotype-by-treatment interaction was significant only for the 100-seed weight in the late group (Supplementary Table 1). Under saline conditions, there was a significant genotypic effect on seed yield, shoot dry weight, pod number, seed number and 100-seed weight across both years and within each phenology group (Table 1). However, for seed yield, the range of variation was narrower in the early than in the late group in 2007–08 (S3 - Supplementary figure). Under non-saline conditions, seed yield in the late group varied significantly among genotypes in 2005–06, whereas seed yield did not vary in 2007-08. In the early group seed yield under non-saline conditions varied in both years, and the range of variation was also limited in the early group. Pod number, seed number and 100-seed weight showed significant genotypic effect across both years and within each phenology group (Table 1).

In the early group, seed yield decreased 24 and 52% under saline conditions in 2005–06 and 2007–08, respectively. In the late group, seed yield did not decrease in 2005–06, but the higher NaCl level used in 2007–08 decreased yield by up to 38%. Salinity had a similar effect on shoot dry weight with a 25% and 48% decrease in the early group and 7% decrease in 2005-06 in the late group. Only in 2007–08 the decrease in the late group was slightly less (23%) than the seed yield decrease (38%). The reduction in seed yield was explained by both a decrease in 100-seed weight and seed number. The magnitude of the decreased 100-seed weight was similar in both groups: it decreased by 19% and 20% in the early group in 2005–06 and 2007–08, respectively, and by 17% and 24% in the late group. Seed number decreased by 5 and 40% in 2005–06 and 2007–08, respectively, in the early group. Surprisingly, in the late group the saline treatment increased seed number by 19% in 2005–06, but the more severe treatment in 2007–08 decreased it by 18%.

The heritability of seed yield in the saline treatment was high in both phenology groups in 2005–06, close to 0.78, although it decreased to about 0.60 the higher salinity used in

2007–08. Heritability was usually higher for seed yield components than for seed yield: heritability for pod number was 0.61 and 0.66 for the early and the late group in 2007–08 while heritability for seed number was up to 0.86 in 2005–06 and heritability changed little in 2007–08 (0.78 and 0.86 for the early and late groups, respectively); heritability for the 100-seed weight was even higher and almost unchanged across the two phenology groups and trial years (Table 1).

Factors affecting the seed yield under saline conditions

Seed yield under non-saline conditions - In neither of the two years nor within the two phenology groups, did seed yield under saline conditions relate to that in non-saline controls (data not shown; in the early group, $R^2 = 0.05$ and 0.06 in 2005–06 and 2007–08, respectively; in the late group, $R^2 = 0.12$ and 0.00 in 2005–06 and 2007–08, respectively). This is different to a previous report (Vadez et al. 2007), but similar to a more recent one (Krishnamurthy et al. 2011), where seed yield under salinity and seed yield were not closely related, and therefore where the seed yield under salinity could not be accountable for the yield potential (seed yield under non-saline control conditions). Because of this lack of relationship between the seed yield under saline conditions and that under non-saline conditions, we have not used the yield ratio (saline seed yield/non-saline seed yield, which would reflect a relative performance under salt stress) that was used in Vadez et al. (2007), nor the seed yield difference between treatments (non-saline seed yield minus saline seed yield, which would reflect how far a genotype is from its non-stressed control). The yield ratio and the yield difference between treatments were closely related ($R^2 = 0.96$ and 0.77 in 2005-06 and 2007-08), but the yield ratio was poorly related to the seed yield under saline conditions, except in one case (2005-06: $R^2 = 0.21$ and 0.55 in the early and late group; 2007-08: $R^2 = 0.06$ and 0.21 in the early and late group). Therefore, seed yield under salinity was used as the measure of salt tolerance in the present study – yield in saline conditions being the objective of breeders (cf. Richards 1983).

Flowering time - Although seed yield under salinity was positively correlated with time to flowering across phenology groups ($R^2 = 0.32$ and 0.59 in 2005–06 and 2007–08,

respectively, polynomial fit not shown), there was a strong clustering of entries by phenology group, especially in 2007–08 when the treatment was more severe (100 mM NaCl) (S3 - Supplementary figure). Within the early group, there was a weak although significant relationship between seed yield and flowering time in both years ($R^2 = 0.08$ and 0.09 in 2005–06 and 2007–08, respectively), with higher seed yield in later entries. Within the late group, there was no relationship between seed yield and flowering time (S3 - Supplementary figure).

Shoot dry weight under saline conditions - Across both phenology groups, seed yield under salinity was significantly related to shoot dry weight under salinity. When the entries were separated by phenology group, this relationship was highly significant in the early group ($R^2 = 0.65$ and 0.67 in 2005–06 and 2007–08, respectively). In contrast in the late group, the relationship between seed yield and shoot dry weight was significant, but with a smaller correlation coefficient in 2005–06 ($R^2 = 0.27$) and not significant in the higher salt treatment in 2007–08 ($R^2 = 0.01$) (Fig. 1).

Seed number under saline conditions - Seed yield under salinity was significantly related to seed number across both phenology groups. After separating the entries by phenology group, this relationship remained highly significant within each group, except for the early group in 2007–08 ($R^2 = 0.16$), (early group, $R^2 = 0.53$ in 2005-06; late group, $R^2 = 0.47$ and 0.46 in 2005–06 and 2007–08, respectively). Figure 2 also separates seed number under salinity between the early and late groups, and shows a higher seed number in the late group compared to the early group (see also Table 1).

100-seed weight under saline conditions - The range of variation for 100-seed weight was similar in both phenology groups (Fig.6). Seed yield under saline conditions had no significant relationship with the 100-seed weight, either across both groups or after separating entries within the two phenology groups, when plotted against 100-seed weight (S4 - Supplementary figure).

Linkage map and QTL analysis

Of the 216 markers tested, 135 markers were mapped on to eight linkage groups spanning a distance of 310.2 cM, although 81 markers remained unmapped. Linkage groups were assigned to chromosomes based on known location of legacy SSR markers (Winter *et al.* 2000, Nayak *et al.* 2010). The number of markers per linkage group ranged from 7 (LG8) to 45 (LG6). The length of each linkage group varied from 5.1 cM (LG2) to 129.9 cM (LG3). Overall inter marker distance was 2.3 cM (Fig. 3). QTLs identified for different surrogate traits under saline and non-saline conditions in both environments are also shown on the map.

While undertaking QTL analysis, no QTL was found for seed yield under salinity in the early phenology group in either year or treatment. However, of the possible components of seed yield in that group, one QTL for shoot dry weight under salinity was found on LG1 in 2007–08, explaining 13% of the variation, and one QTL for seed number under salinity was found on LG7 in 2007–08, explaining 25% of the variation (Table 2). No QTLs were detected for yield ratio among early phenology group.

In the late phenology group, a QTL was found for seed yield under salinity on LG3 in 2007–08, explaining 19% of the variation. In the late group, one QTL was also found on the same linkage group under non-saline control conditions, although in a different genomic region, for shoot dry weight in 2007-08 and for yield in 2005–06 and 2007–08 (Table 2; S5 – Supplementary figure). Among the surrogates for seed yield under salinity, a genomic region was identified on LG6 that contained QTLs for pod number, seed number, 100-seed weight under salinity as well as non-saline (control) conditions in 2007–08. The QTL for pod number under salinity in this genomic region explained as much as 37% of the phenotypic variation. Similarly, QTLs for seed number and 100-seed weight were found under both saline and non-saline conditions during 2005–06 in the same genomic region on LG6, where QTLs for pod number, seed number, and 100-seed weight under saline and non-saline conditions in 2007–08 were found (Table 2; S6 and S7 – Supplementary figures). One QTL for flowering time was also found consistently across treatment and year of experiment, located on LG4 (S6 – Supplementary figure). This QTL was flanked by three SSR markers TA35, TA144 and TS57 and explained 18.5–34.4% of the phenotypic variation in flowering time. One QTL was found for the yield ratio in 2007-08 and contributed a phenotypic variance of 34.6%.

When the phenotyping data were used for QTL analysis, disregarding the groups of phenology, no QTLs for seed yield and yield ratio was found in any of the treatments for either of the two years. Nevertheless, a genomic region containing QTLs for seed number and 100-seed weight under saline conditions in both 2005–06 and 2007–08 was found on LG6. The same genomic region also contained QTLs for seed number and 100-seed weight under non-saline conditions in both years (S7 – Supplementary figure).

Discussion

A large range of variation for seed yield under salinity was found within each of the two phenology groups of a RIL population segregating for flowering time. In both groups, high pod and seed numbers under saline conditions appeared to be the most important traits for higher seed yield. Also, within groups of phenology there was no relationship between the time to flowering, or the 100-seed weight, and seed yield. The present data for this RIL population, together with earlier results obtained for a set of chickpea lines of diverse backgrounds with a larger range of flowering times, but also presumably differing in many other traits (Vadez et al. 2007), shows that time to flowering was not a major determinant of yield under the saline conditions imposed, since seed yield under salt stress and flowering time were not (late group), or very weakly (early group), related within maturity groups. In addition, seed yield in the present study was also related to shoot dry weight in the early phenology group, a relationship not present in a wider germplasm set (Vadez et al. 2007). Several QTLs were identified for seed yield and its components under saline conditions within each phenology group, with limited overlap, but no major QTL was identified when the analysis was carried out on the entire set of this RIL population.

Traits related to salt tolerance

Contrary to previous data on responses of a diverse set of chickpea genotypes to salinity (Vadez et al. 2007), the present study of RILs found no significant relationship between seed yield under salinity and seed yield under control treatment. This finding was

presumably related to the relative earliness of the genotypes tested here, which all flowered in less than 55 days and were well adapted to the short season environment in which these were tested; flowering times in previous work ranged from 30 to 100 days (Vadez et al. 2007). Since the seed yield under saline condition was unrelated to the seed yield under control, in the present case the absolute seed yield under saline conditions was the preferred measure of salt tolerance, rather than the ratio of seed yield (seed yield saline conditions / seed yield non-saline conditions). This ratio was in fact poorly related to the seed yield under saline conditions and this reflects that the genotypic expression of seed yield under salt stress is independent from the yield potential (yield under non-saline conditions) and is specific to the stress conditions. Therefore, the use of the yield ratio in this case would be less informative than the yield *per se* in saline conditions, towards our eventual goal of breeding for improved yield in saline soils (cf. Richards 1983).

Salinity tolerance, measured here as seed yield under salinity, was then strongly related to seed number, in both the entire genotype set and in the two separate phenology groups. In contrast, there was no relationship between salinity tolerance and the ability of genotypes to fill seeds (seed size, measured by the 100-seed weight). This confirms previous data (Vadez et al. 2007; Krishnamurthy et al. 2011) and extends the validity of the hypothesis that for genotypes with relatively early duration, salinity tolerance in chickpea is dependent on successful production of reproductive sites under salt stress, but the present work also found an association also with biomass in the early group (discussed in the next paragraph). Other reports also point to reproduction as the most sensitive process in chickpea under salt-stress (Mamo et al. 1996; Katerji et al. 2001; Datta et al. 1987; Samineni et al. 2011) and the reproductive phase is also sensitive to drought (Leport et al. 1999, 2006). Detailed investigations are ongoing to better understand the process(es) affected during reproduction.

An interesting difference to previous work (Vadez et al. 2007) was the significant relationship between seed yield and shoot dry weight in the 'early' group of entries. Serraj and colleagues (2004) reported a 60% reduction in shoot biomass under similar saline conditions in a set of 252 genotypes. Reduced shoot biomass may be deleterious for early flowering lines that do not accumulate significant biomass before flowering, and where only a small delay in flowering time under saline conditions could not help

compensate. Salinity may reduce branching in early flowering lines and thus reduce the number of possible floral nodes (Saxena 1984). This may be reflected in a lower shoot dry weight, which was in fact the main factor explaining the associated yield reduction in the early entries in the RIL population assessed here. In 2007–08, the seed number also decreased significantly in the early group, but the reduction in shoot weight was even larger. We tested whether seed number was related to shoot dry weight in the early entries, but found only a weak relationship ($R^2 = 0.12$ in 2005–06 and 0.04 in 2007–08). These data indicate that in early entries high shoot biomass and seed number both contribute to determining high seed yield under salinity: salt tolerant early lines appear to be capable of developing high shoot biomass with possibly more floral nodes, and ensuring reproductive success in a large number of those floral nodes. The higher percentage decrease in shoot biomass in the early entries than the percentage decrease in seed number may indicate that the early entries suffered more as a result of the salinity from a reduction in biomass production than from a reduction in successful reproductive sites.

Clearly, salinity affected the short duration lines more than longer duration lines, and the effect was due to both reduced biomass production and seed numbers in the early entries. Seed number increased under salinity in 2005–06 in the late group, and was the trait best correlated to seed yield ($R^2 = 0.53$), whereas shoot biomass was decreased 7%. This was surprising considering that reduced flower numbers in stressed plants are generally reported, e.g. chickpea (Nayyar et al. 2005; Leport et al. 1998). However, there have been earlier reports of an increase in flower number in chickpea with low/moderate salinity treatments (Dhingra and Varghese 1993; Samineni et al. 2011). Also, earlier reports indicate that later entries tend to produce more flowers than early entries under salt stress (Katerji et al. 2001). So, in the late group, the capacity to produce more flowers under salt stress could have given an additional benefit to these entries, even despite a slight decrease in shoot biomass. In the late group, shoot biomass was not related to seed yield, which might be explained by the fact that late entries had more days to accumulate resources before flowering and also flowering time was delayed 4 and 5 days under saline conditions. This observation of delayed flowering in saline conditions contrasts with earlier onset of flowering under terminal drought than under fully irrigated conditions

reported in chickpea (Krishnamurthy et al. 1999). The delay in saline conditions might involve hormonal regulation (e.g. ABA), as increased ABA has previously been reported to delay flowering (Achard et al. 2006).

QTL analysis – Linkage mapping and QTL analysis

The intraspecific map, based on ICCV 2 × JG 62, spanned 310.2 cM; the number of markers mapped and length of linkage groups was not correlated. For instance, although 22 markers were mapped on both LG1 (8.9 cM) and LG3 (129.9 cM), the length of linkage groups varied significantly (Fig. 3). Similar results have been reported by Radhika et al. (2007) and Nayak et al. (2010). Uniform marker distribution was not observed in LG3, LG5 or LG8. The uneven distribution of markers on linkage groups may be due to unequal recombination events in these chromosomal regions.

One major finding of this work was a QTL for seed yield, found specifically in the late group, on LG3 and explaining a substantial portion of the phenotypic variation (19%). This is the first ever reported QTL for salinity tolerance in chickpea. Other than chickpea, there are not many reports in other crops dealing with identification of QTLs for salinity tolerance, and most are QTLs for traits such as sodium exclusion in rice (Ren et al. 2005) or growth (Takehisa et al. 2004). Fewer studies again have identified QTLs for seed yield under stress: barley (Ellis et al. 2002), soybean (Lee et al. 2004), wheat (Quarrie et al. 2005) and rice (Gregorio et al. 2002).

In the present study, QTLs for seed yield were only found in the late flowering lines, not in the early flowering lines. However, a QTL for shoot dry weight was found in the early group, explaining a minor percentage of the variation. Combined QTL analysis of the entire RIL population did not reveal any QTLs, highlighting the importance of first elucidating the role of phenology in the genotypic response to salt stress. QTLs for yield components explained a large proportion of the phenotypic variation, justifying their possible use in breeding programs. A genomic region on LG6 (Fig. 3), harboring many QTLs for different salinity-tolerance-related traits such as seed number and 100-seed weight, in both early and late phenology groups under saline and non-saline conditions, was identified across the two years and treatments. These QTLs explained about 14.8–

49.7% of the phenotypic variation for different surrogate traits. This genomic region is believed to harbor genes governing seed yield, which seem to be closely related to constitutive trait governing seed number or seed development, since this genomic region was also identified under non-saline control conditions (Table 2). This is also in agreement with the absence of a strong (2005-06) or of a significant (2007-08) genotype-by-treatment interaction for these traits in the late group (Supplementary Table 1). Similarly, a genomic region on LG4 harboring QTLs for salinity-tolerance-related traits like days to 50% flowering, seed number and shoot dry weight explained about 8.8–37.7% of the phenotypic variation. These two genomic regions harboring many QTLs with higher phenotypic variation, after validation, may serve as potential candidate regions for trait improvement through marker-assisted backcrossing (MABC) (see Varshney et al. 2007, 2009). In any case, QTLs for pod or seed number always explained a larger percentage of the phenotypic variation than QTL for shoot dry weight. Only one major QTL with 34.6% phenotypic variation was found on LG6 for yield ratio during 2007-08 environment, although, as expected, this QTL had no relationship with the cluster of QTLs on LG6 for salinity tolerance surrogates such as seed number or pod number.

Conclusion

This is the first report on QTLs for seed yield and components under salinity stress in chickpea. It confirms that salinity tolerance in chickpea is closely related to the success of reproduction under stress, but also points to an additional/independent tolerance mechanism, related to shoot biomass development, in early flowering genotypes. These earlier-flowering entries, in which seed yield under stress was related to both shoot biomass and seed number, were more sensitive to salinity than later-flowering entries where only seed number correlated with seed yield under salinity.

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Figure captions

Fig. 1 Relationship between seed yield and shoot dry weight in saline conditions in 2005–06 (a) and 2007–08 (b) within two phenology groups, i.e. early group (flowering within 38 days after sowing in saline conditions, open symbols) and late group (flowering 41 days or more after sowing in saline conditions, closed symbols). Data are means of four replicate pots per genotype with four plants per replicate pot. Lines are fitted linear regressions.

Fig. 2 Relationship between seed yield and seed number in saline conditions in 2005–06 (a) and 2007–08 (b) within two phenology groups, i.e. early group (flowering within 38 days after sowing in saline conditions, open squares) and late group (flowering 41 days or more after sowing in saline conditions, solid squares). Data are means of four replicate pots per genotype with four plants per replicate pot. Lines are fitted linear regressions.

Fig. 3 Genetic linkage map of chickpea (ICCV 2 × JG 62) with 135 marker loci on eight linkage groups. Kosambi map distances are on left hand side, genomic regions harboring QTLs (black bars) and QTLs for salinity-related-traits (colored squares), as listed in Table 2, on right hand side of linkage group for early phenology group (E), late phenology group (L), under both saline (S) and non-saline (C) conditions and two environments 2005–06 (5) and 2007–08 (7).

S1 - Supplementary figure 1. Frequency distribution of flowering time in the (a) non-saline control treatment and (b) saline treatment in 126 F12 RIL progenies, plus parental lines, of the cross between ICCV 2 and JG 62. Within each treatment, flowering time for each line was the average flowering time for the two years.

S2 - Supplementary figure 2. Relationship between mean flowering time under non-saline control conditions and in the saline treatment for the RILs and parental genotypes. The eight genotypes from the early flowering group that have delayed flowering under

salt treatment are ringed by an ellipse. Within each treatment, flowering time for each line was the average flowering time for the two years. The solid line is the fitted linear regression and the dashed line is the 1:1 line.

S3 - Supplementary figure 3. Relationship between flowering time and seed yield under saline conditions each year (2005–06, circles; 2007–08, squares) for genotypes in the early flowering group (flowering within 38 days after sowing in saline conditions, open symbols) and late flowering group (flowering 41 days or more after sowing in saline conditions, solid symbols). Data are means of four replicate pots per genotype with four plants per replicate pot. Lines are fitted regressions.

S4 - Supplementary figure 4. Scatter plot exploring the relationship between seed yield and the 100-seed weight in saline conditions in 2005–06 (a) and 2007–08 (b) within two phenology groups, i.e. early group (flowering within 38 days after sowing in saline conditions, open squares) and late group (flowering 41 days or more after sowing in saline conditions, solid squares). Data are means of four replicate pots per genotype with four plants per replicate pot.

S5 - Supplementary figure 5. Linkage group 6 that shows QTLs for seed number and 100-seed weight under saline conditions and non-saline conditions in both 2005-06 and 2007-08.

S6 - Supplementary figure 6. A snapshot showing stability of the QTL for flowering time across treatments (saline and non-saline conditions) and environments (two years).

S7 - Supplementary figure 7. A snapshot showing stability of the QTL for seed number under non-saline condition across the two environments (i.e. two years 2005-06 and 2007-08).

Table 1 One-way ANOVA probabilities (F-Prob), means and heritabilities (h^2) for days to flowering, seed yield (g pot⁻¹), shoot dry weight (DW, g pot⁻¹), pod number (pot⁻¹), seed number (pot⁻¹) and 100-seed weight (g) for 'early' genotypes (flowering time <38 DAS) and 'late' genotypes (flowering time >41 DAS) in saline and non-saline (control) treatments in 2005–06 and 2007–08.

| | | Salinity | | | | | Control | | | | | | |
|---------|----------------|----------------------|------------|----------|---------------|----------------|--------------------|----------------------|------------|----------|---------------|----------------|--------------------|
| | | Days to flowering | Seed yield | Shoot DW | Pod number | Seed number | 100-seed weight | Days to flowering | Seed yield | Shoot DW | Pod number | Seed number | 100-seed weight |
| 2005–06 | | | | | | | | | | | | | |
| Early | F-Prob | <0.001 | <0.001 | <0.001 | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | | <0.001 | <0.001 |
| | Mean | 38.37 | 8.97 | 21.00 | | 52.06 | 17.85 | 35.305 | 11.68 | 27.96 | | 54.65 | 21.94 |
| | H ² | 0.924 | 0.780 | 0.799 | | 0.866 | 0.907 | 0.960 | 0.688 | 0.734 | | 0.792 | 0.892 |
| Late | F-Prob | <0.001 | <0.001 | <0.001 | | <0.001 | <0.001 | <0.001 | <0.001 | 0.036 | | <0.001 | <0.001 |
| | Mean | 50.03 | 11.38 | 29.18 | | 62.76 | 18.98 | 45.54 | 11.51 | 31.43 | | 52.71 | 22.77 |
| | H ² | 0.943 | 0.776 | 0.790 | | 0.867 | 0.907 | 0.938 | 0.814 | 0.592 | | 0.915 | 0.857 |
| 2007–08 | | | | | | | | | | | | | |
| Early | F-Prob | <0.001 | 0.013 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.002 | <0.001 | 0.010 | <0.001 | <0.001 |
| | Mean | 31.18 | 5.28 | 11.78 | 33.31 | 35.29 | 15.44 | 32.64 | 10.85 | 22.43 | 52.70 | 59.00 | 19.25 |
| | H ² | 0.938 | 0.605 | 0.763 | 0.716 | 0.776 | 0.928 | 0.802 | 0.635 | 0.654 | 0.610 | 0.728 | 0.921 |
| Late | F-Prob | <0.001 | <0.001 | 0.006 | <0.001 | <0.001 | <0.001 | <0.001 | 0.184 | 0.060 | <0.001 | <0.001 | <0.001 |
| | Mean | 46.95 | 7.66 | 22.30 | 49.31 | 52.35 | 15.43 | 44.86 | 11.98 | 28.29 | 55.50 | 62.40 | 20.08 |
| | H ² | 0.918 | 0.624 | 0.753 | 0.837 | 0.864 | 0.897 | 0.841 | 0.545 | 0.578 | 0.656 | 0.650 | 0.928 |

Table 2: Percentage of variation explained by QTLs identified for days to flowering (DF), shoot dry weight (SDW), seed yield (SYLD), pod number (PN), seed number (SN), yield ratio(YLD_R), yield difference (YLD_D) and 100-seed weight (100SW) for 'early' genotypes (flowering time <38 DAS), 'late' genotypes (flowering time >41 DAS), and both groups together in saline and non-saline (control) treatments in 2005–06 and 2007–08

| | | Trait | Early genotypes | | | | Late genotypes | | | | All genotypes | | | |
|---------|---------|-------|-----------------|-----------------|-----|--------------|----------------|-----------------|-----|--------|---------------|-----------------|-----|--------|
| | | | LG | Marker interval | LOD | PV (%) | LG | Marker interval | LOD | PV (%) | LG | Marker interval | LOD | PV (%) |
| 2005-06 | Saline | DF | 4 | TA144 – NO_Y_13 | 2.6 | 13.2 | 4 | TA144 – NO_Y_13 | 3.1 | 24.5 | 5 | TA114 – TA78 | 3.4 | 13.8 |
| | | SDW | - | - | - | - | - | - | - | - | 4 | TA127 – TS57 | 2.5 | 8.8 |
| | | SN | - | - | - | - | 6 | TR20s – TA46 | 3.3 | 25.1 | 6 | TR20s – TA46 | 2.6 | 21.1 |
| | | 100SW | 7 | TA11 – TA42 | 6.2 | 27.6 | 6 | TA186 – TA46 | 4 | 23.3 | 6 | TR20s – TA46 | 3.4 | 21.4 |
| | | 100SW | - | - | - | - | 7 | TR59 – TS53 | 2.8 | 17.6 | - | - | - | - |
| | | HI | 7 | TA11 – TA42 | 2.9 | 15.1 | 6 | TA186 – TA46 | 2.7 | 15.2 | 6 | TR20s – TA46 | 2.5 | 11.1 |
| | Control | HI | 2 | TA200 – TA37 | 2.5 | 11.9 | - | - | - | - | 5 | TA114 – NO_X_1 | 3.1 | 11.5 |
| | | DF | 4 | TA127 – TS57 | 6.2 | 15.8 | 4 | TA144 – NO_X_1 | 5.8 | 37.7 | 3 | TA106 – Podnode | 2.5 | 10.1 |
| | | SN | - | - | - | - | 4 | TA144 – NO_Y_13 | 3.7 | 17.2 | - | - | - | - |
| | | | - | - | - | - | 6 | TA186 – TA46 | 3.9 | 15.1 | - | - | - | - |
| | | 100SW | 6 | TR20s – TA46 | 6.4 | 40.7 | 6 | GA137 – TA46 | 4.6 | 49.7 | 6 | GA137 – GA25 | 2.5 | 18.1 |
| | | | 6 | TR20s – TA46 | 3.5 | 25.6 | 2 | TA200 – TA37 | 3.7 | 18.3 | - | - | - | - |
| | | HI | 7 | TA11 – TA42 | 5.4 | 21.4 | 6 | TA186 – TA46 | 5.3 | 20.4 | 6 | TR20s – TA46 | 5.3 | 32.3 |
| SYLD | - | - | - | - | 3 | TA14s – TR40 | 2.9 | 22.4 | - | - | - | - | | |

| | | | | | | | | | | | | | | |
|---------|---------|-------|---|--------------|-----|------|---|--------------------|-----|------|---|--------------|-----|------|
| 2007-08 | Saline | DF | - | - | - | - | 4 | TA186 - TA46 | 3.3 | 18.5 | - | - | - | - |
| | | SDW | 1 | TA203 – TR42 | 3.4 | 13.3 | 5 | TS46 – NO_X_1 | 2.7 | 26.6 | 5 | TA114 – TA78 | 4.9 | 19.5 |
| | | SN | 7 | TA11 – TA42 | 4.8 | 24.7 | 6 | opng11 – TA46 | 2.9 | 15.7 | 6 | TR20s – TA46 | 2.7 | 12.3 |
| | | 100SW | - | - | - | - | 6 | GA137 – GA25 | 3.2 | 43.2 | 6 | TR20s – TA46 | 3 | 17.3 |
| | | HI | - | - | - | - | 6 | TA186 – TA46 | 3.4 | 18.2 | - | - | - | - |
| | | SYLD | - | - | - | - | 3 | TA106 – Podnode | 3.2 | 19.2 | - | - | - | - |
| | | PN | - | - | - | - | 6 | GA137 – TA46 | 3.9 | 37.2 | 7 | TA11 – TA42 | 2.5 | 7.7 |
| | Control | DF | 3 | TA14s – TR40 | 3.5 | 13.6 | 4 | TA35 – TS57 | 4.2 | 24.5 | 5 | TA114 – TA78 | 3.6 | 12.6 |
| | | SDW | - | - | - | - | 3 | TA196 – TA96 | 2.9 | 55.6 | - | - | - | - |
| | | SN | 6 | TR20s – TA46 | 2.7 | 27.5 | 6 | TA186 – TA46 | 2.6 | 14.8 | 6 | GA137 – TA46 | 3.1 | 18.5 |
| | | HI | - | - | - | - | 4 | TA127 – TS57 | 2.5 | 12.7 | - | - | - | - |
| | | 100SW | - | - | - | - | 6 | TA186 – TA46 | 6.5 | 36.6 | 6 | TR20s – TA46 | 6.9 | 28.4 |
| | | HI | - | - | - | - | 4 | TA35 – TS57 | 2.6 | 16 | - | - | - | - |
| | | | - | - | - | - | 6 | TA186 – TA46 | 2.5 | 14.5 | - | - | - | - |
| | | SYLD | - | - | - | - | 3 | Opni18a – TA22 | 2.6 | 16.9 | - | - | - | - |
| | | PN | - | - | - | - | 6 | TA46 – TA132 | 3.7 | 21.3 | - | - | - | - |
| | 2005 | YLD_R | | - | - | - | - | - | - | - | - | - | - | - |
| | | YLD_D | - | - | - | - | - | - | - | - | - | - | - | - |
| | 2007 | YLD_R | - | - | - | - | 6 | TA46 – TS24 | 5.4 | 34.6 | - | - | - | - |
| YLD_D | | - | - | - | - | - | - | - | - | - | - | - | - | |

Supplementary Table 1. Two-way Anova analysis of days to flowering, seed yield (g pot⁻¹), shoot dry weight (g pot⁻¹), seed number (pot⁻¹) and 100-seed weight (g) in two years of data after separating entries according to their flowering time (early entries with flowering time < 38 DAS and late entries with flowering time > 41 DAS).

| | | Days to flowering | Seed Yield | Shoot DW | Pod number | Seed number | 100-seed weight |
|--------------|---------------|--------------------------|-------------------|-----------------|-------------------|--------------------|------------------------|
| Group | F-Prob | 2005-06 | | | | | |
| Early | T | <.001 | <.001 | <.001 | - | 0.007 | <.001 |
| | G x T | <.001 | <.001 | <.001 | - | <.001 | 0.330 |
| Late | T | <.001 | 0.444 | <.001 | - | <.001 | <.001 |
| | G x T | 0.030 | <.001 | 0.003 | - | <.001 | 0.043 |
| | | | | | | | |
| | | 2007-08 | | | | | |
| Early | T | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| | G x T | 0.081 | 0.046 | 0.050 | 0.657 | 0.005 | <.001 |
| Late | T | <.001 | <.001 | <.001 | 0.004 | <.001 | <.001 |
| | G x T | 0.721 | 0.565 | 0.262 | 0.252 | 0.711 | <.001 |

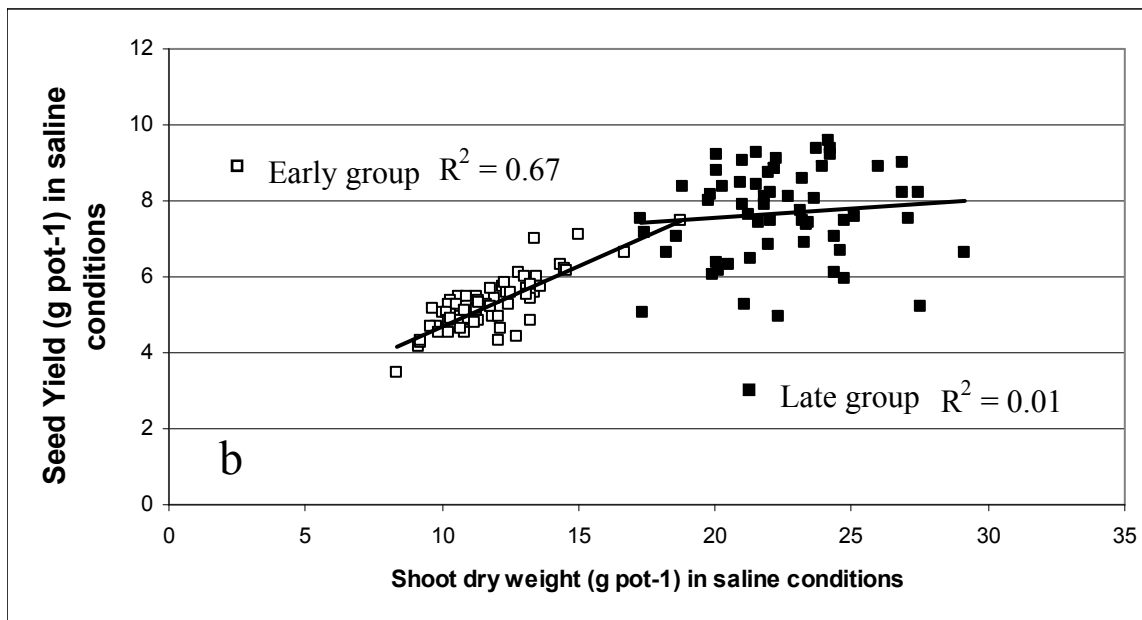
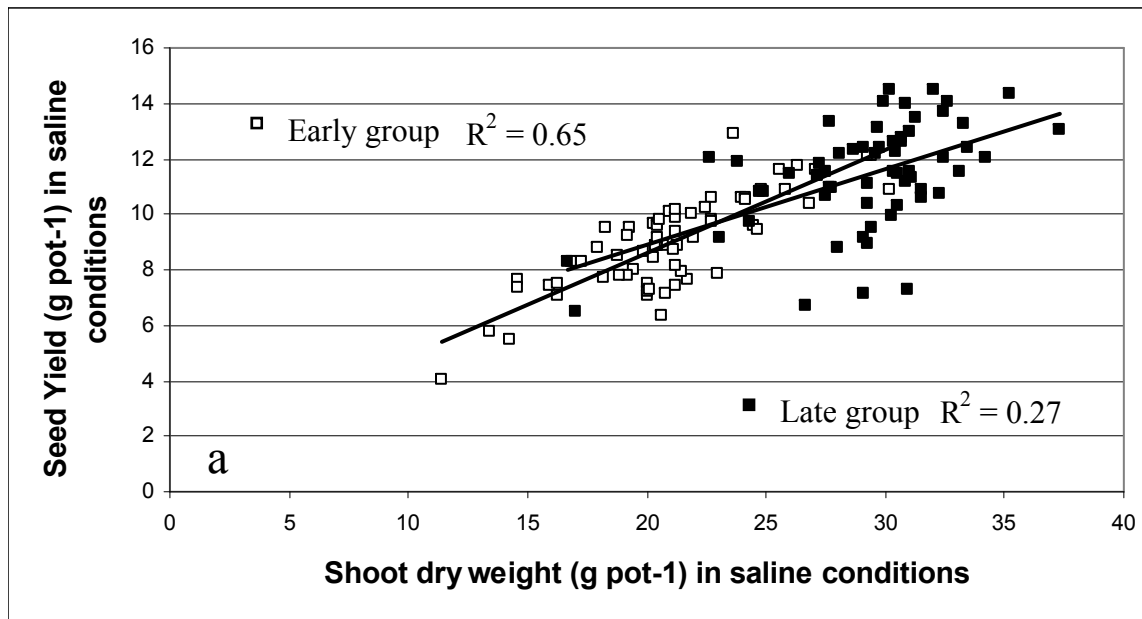


Fig. 1

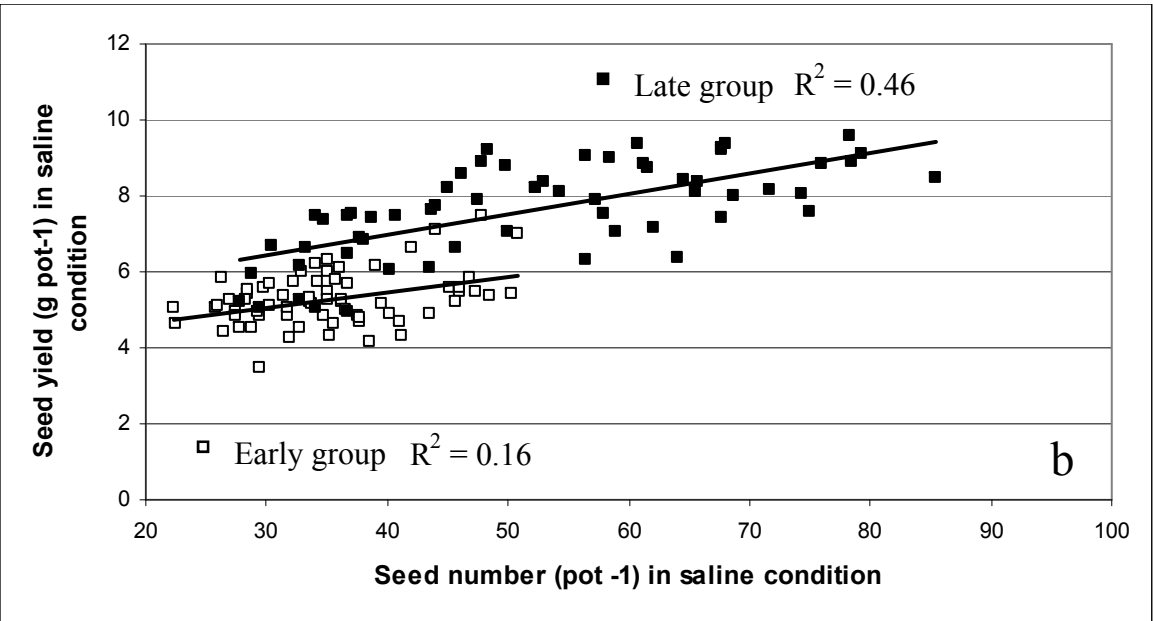
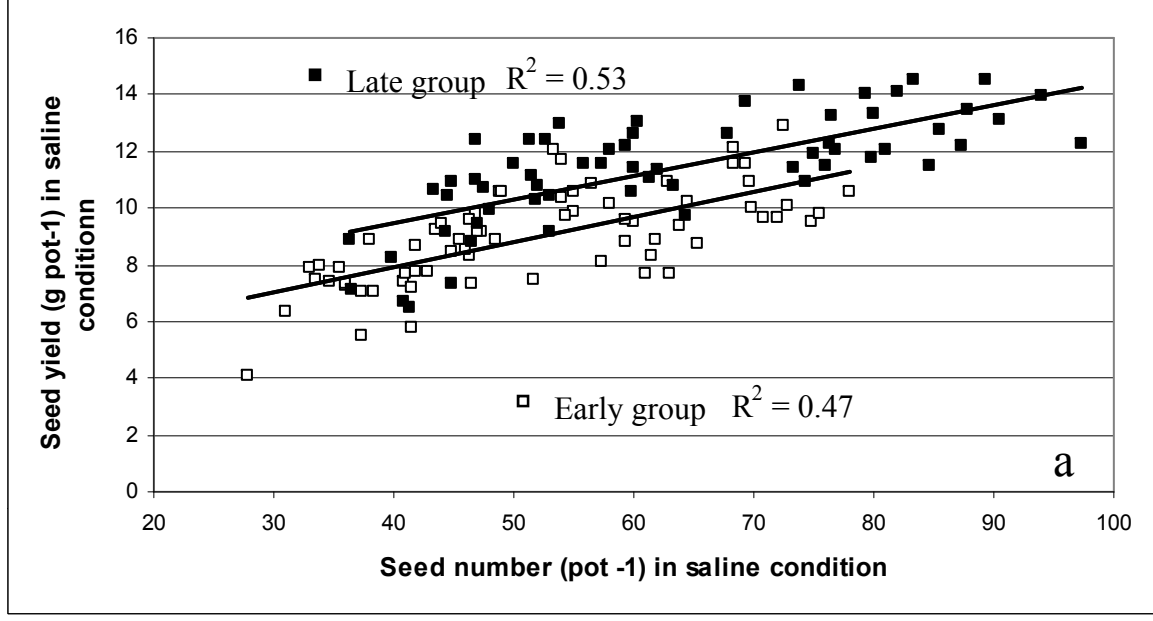
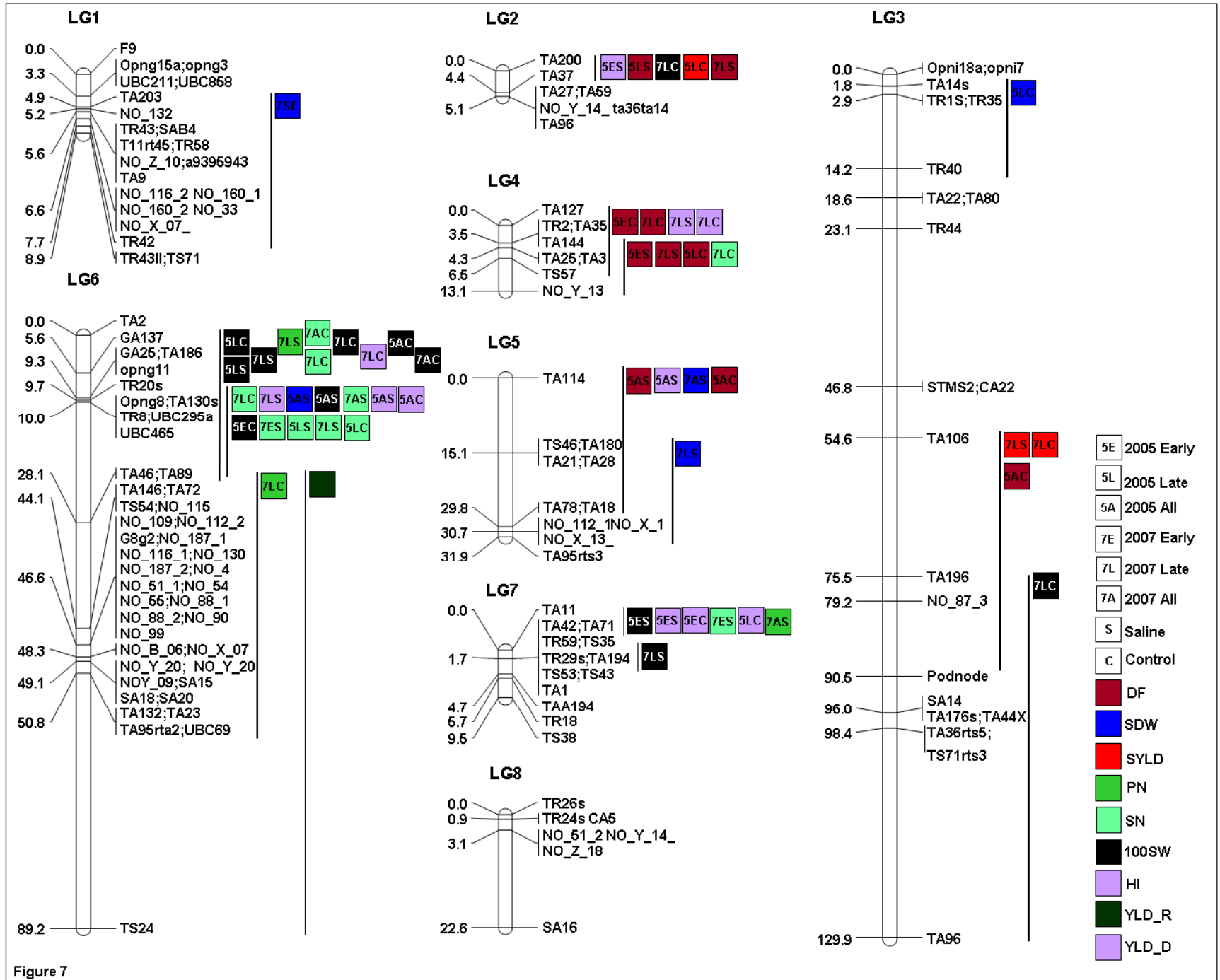
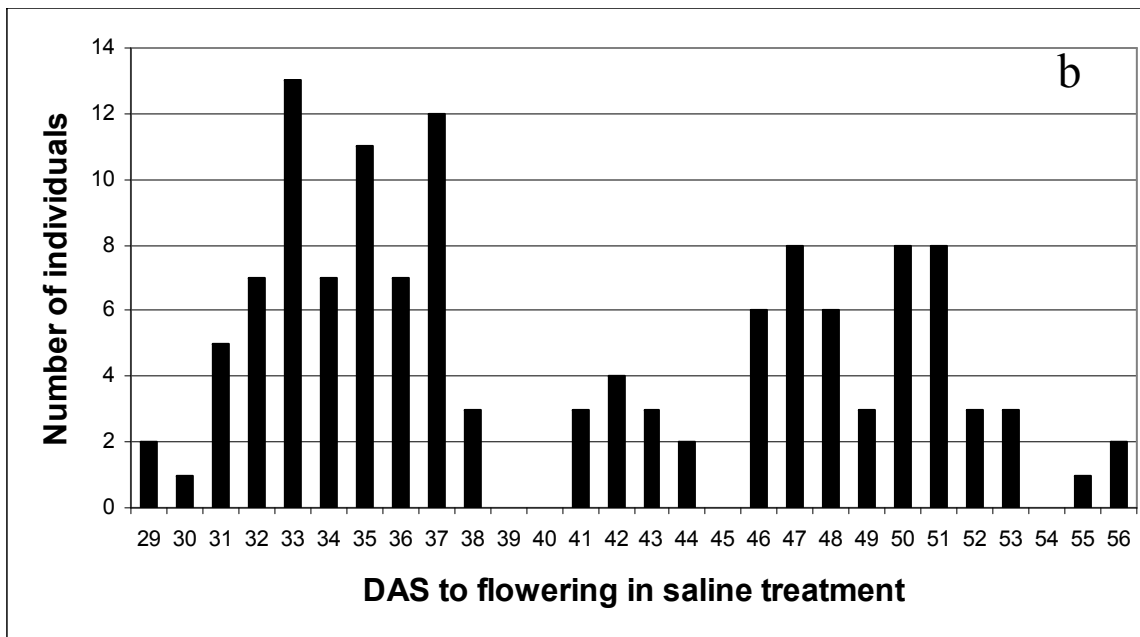
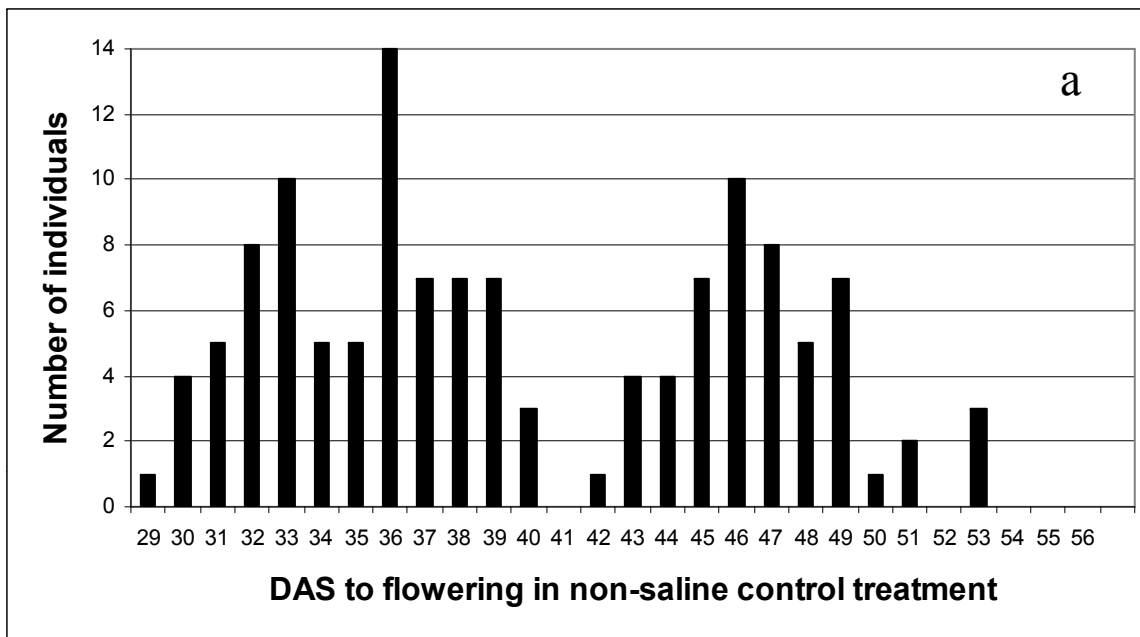


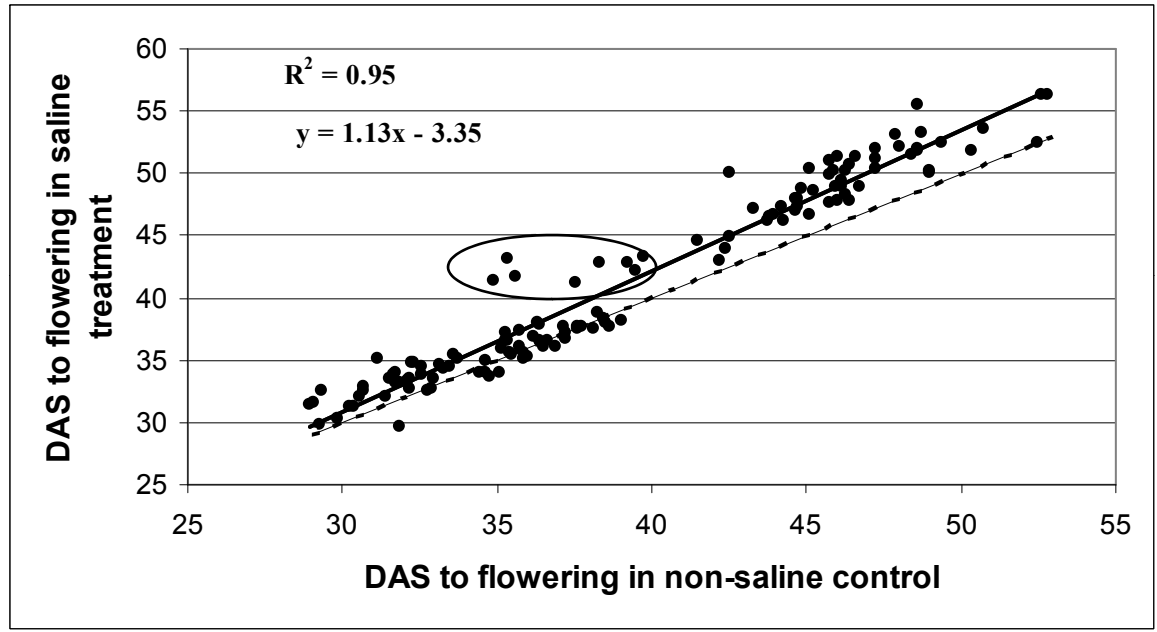
Fig. 2

Fig.3

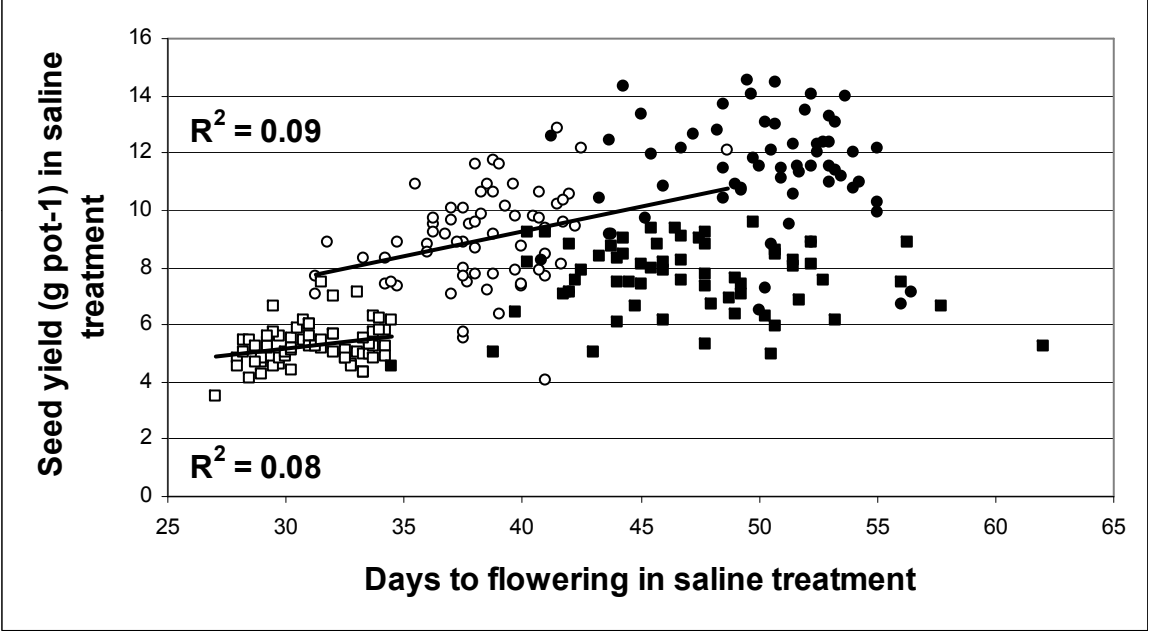


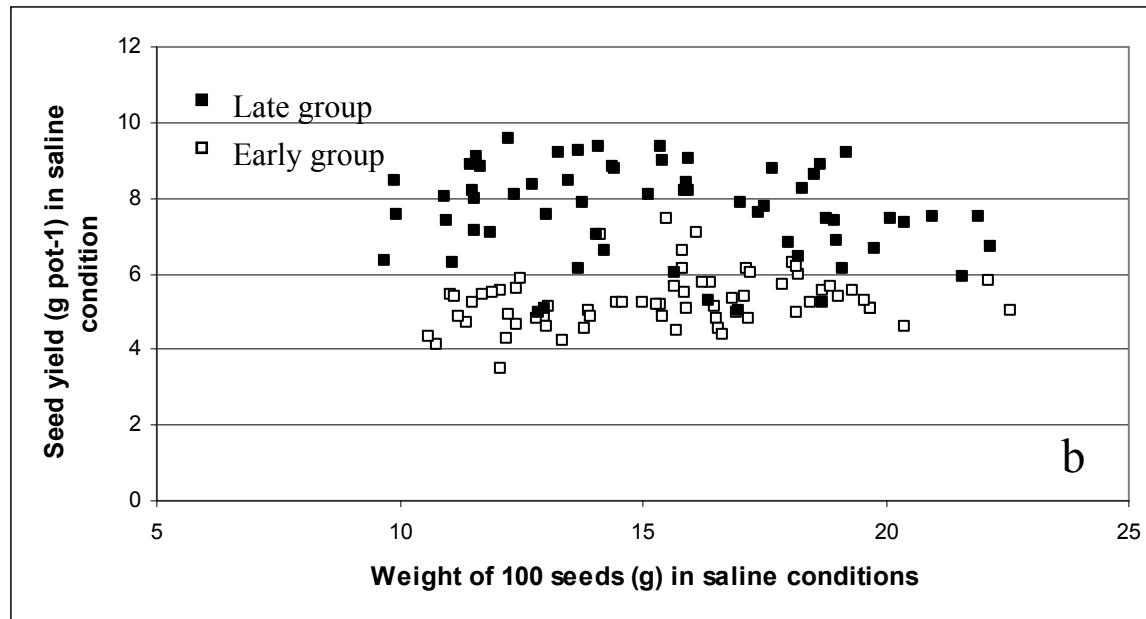
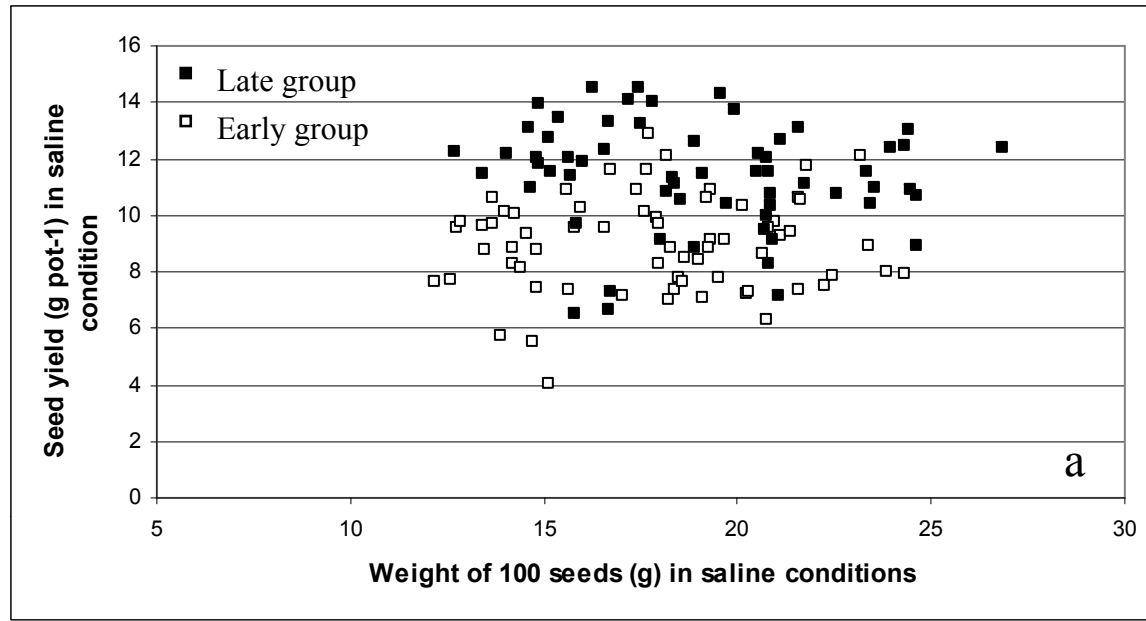
S1





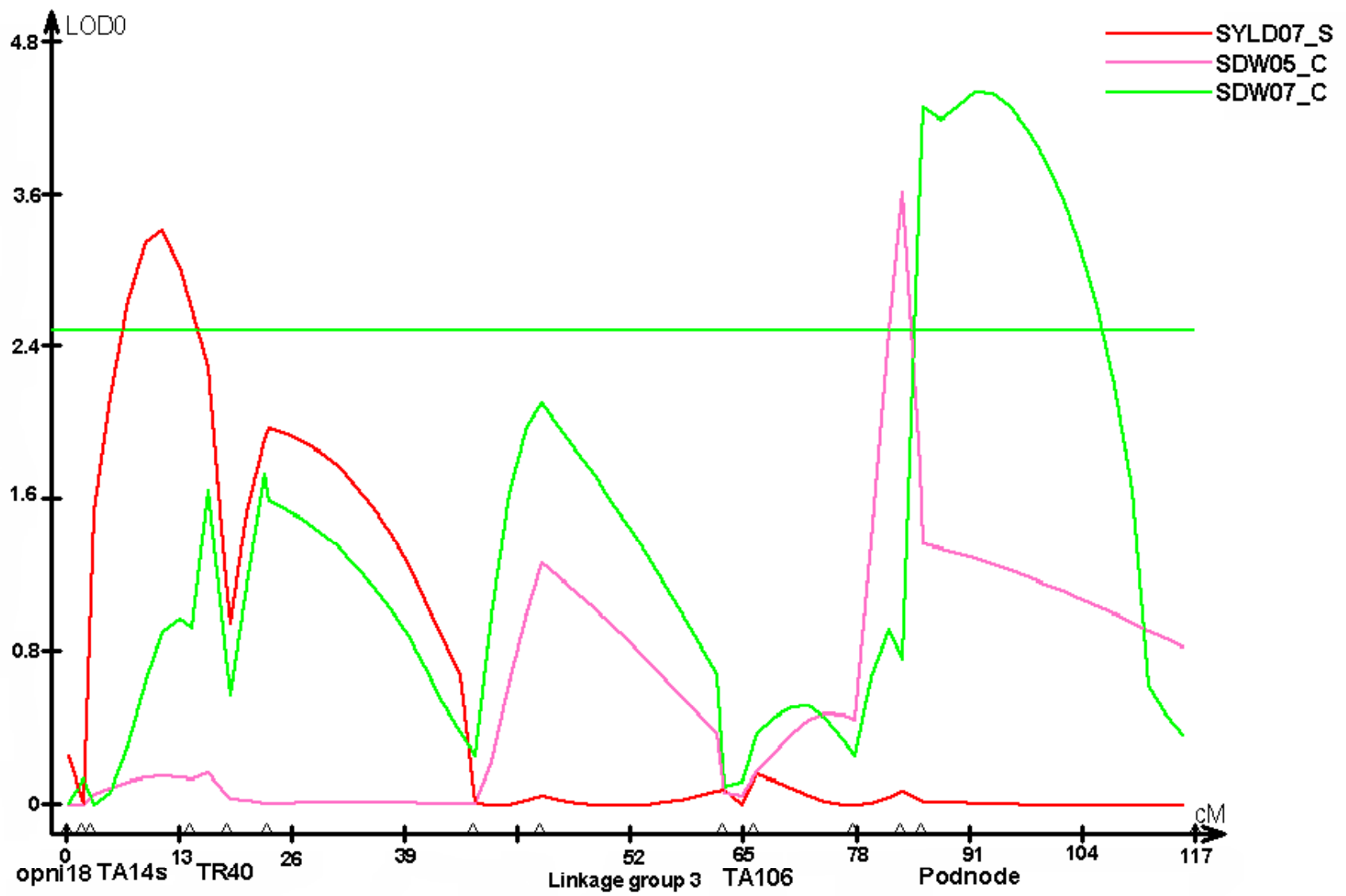
S2



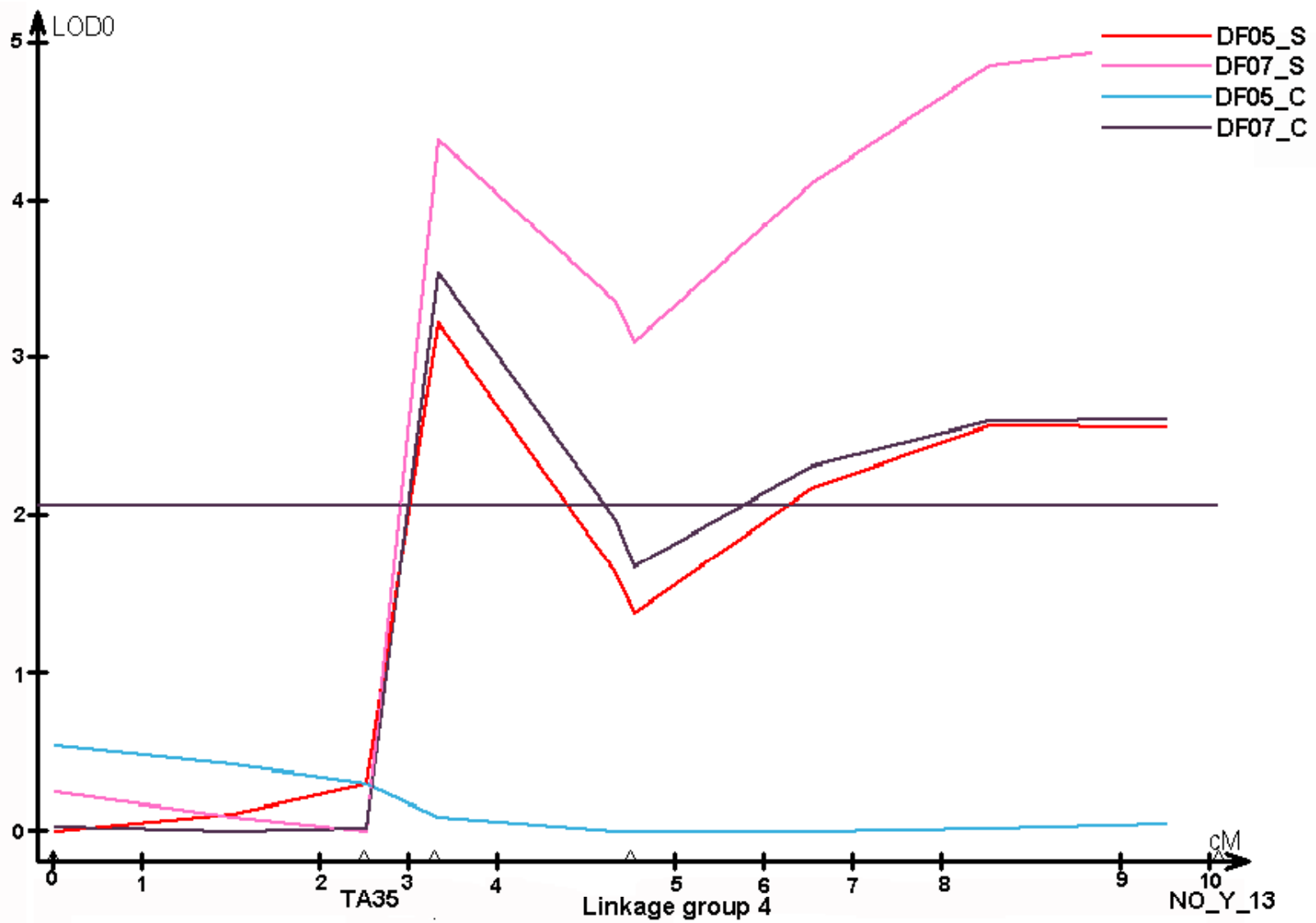


S4

S5

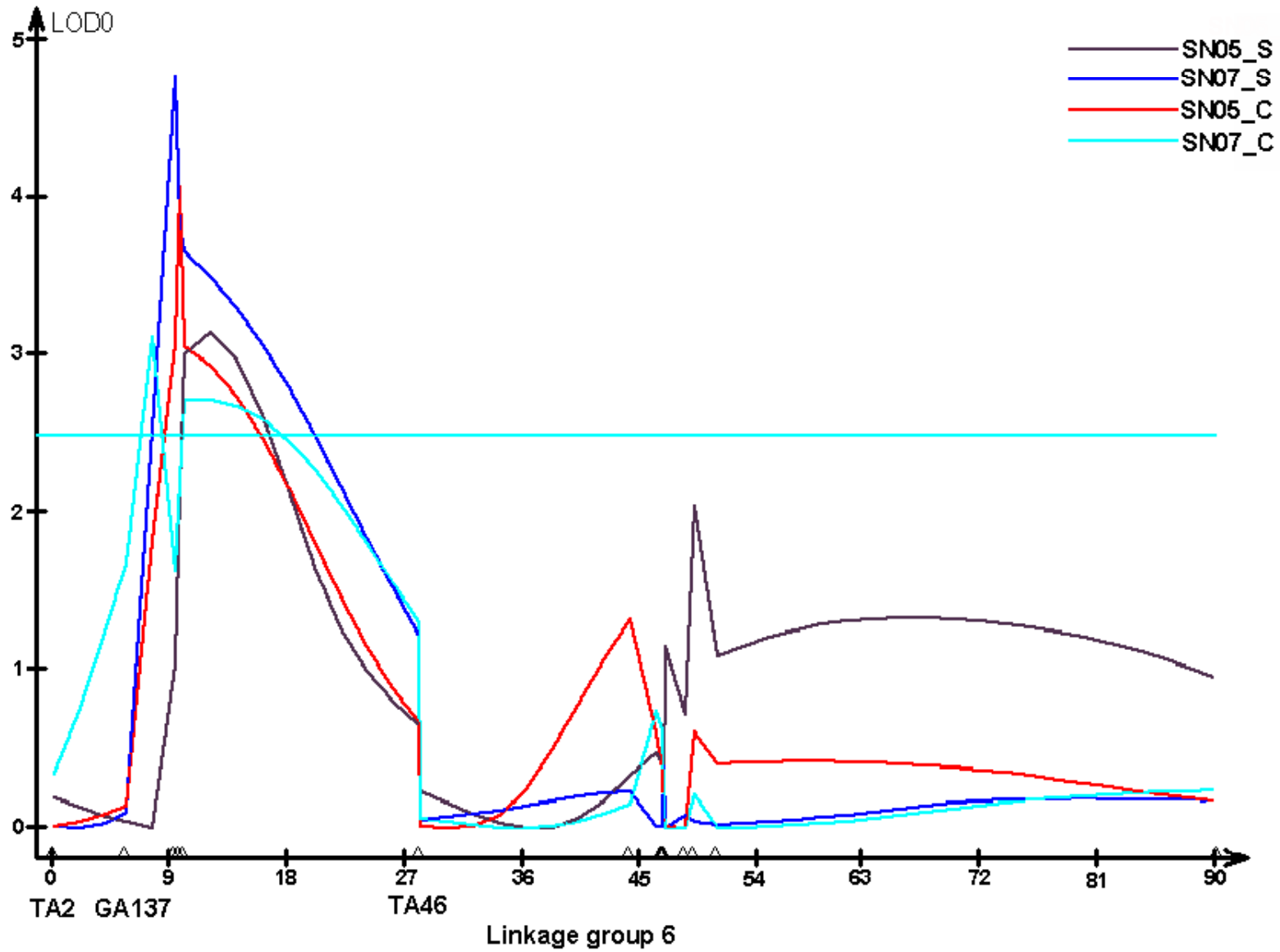


S6



Supplementary 2

S7



Supplementary 3