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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

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cluster of QTL 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 QTL for seed yield and seed number in chickpea exposed to salinity.

Keywords 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) because of 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 to be 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 (QTL) 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 line (RIL) progenies and its relation to their segregation for flowering time. A second question is whether QTL 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). These 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 QTL 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 QTL 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, 2005–2006 and 2007–2008. 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 2005–2006, and 29.8 and 13.9°C in 2007–2008.

In 2005–2006, the saline treatment had 8.77 g NaCl per pot (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 per pot = 25% w/w). In 2007–2008, salt application was increased to 10.96 g NaCl per pot (equivalent to 1.46 g NaCl kg^{-1} soil) to increase the discrimination between entries. In 2007–2008, the treatment was applied in two half-doses (equivalent to 5.48 g NaCl per pot each time), as 1.875 l of a 50 mM NaCl solution at sowing and 1.0 l of a 94 mM NaCl 2 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 amounts 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 of 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/QTL 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 two of four plants flowering) was recorded for 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 per pot), pod weight (g per pot), seed weight (g per pot), 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 the 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 and Green 1987) and the Kosambi mapping function (Kosambi 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 1,000 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. A one-way ANOVA was then 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_g^2 and σ_e^2, were calculated, from which heritability was estimated as $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2)$.

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, respectively), 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 (Electronic Supplementary Material Figure S1).

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 (Figure S2). 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) (Figure S1).

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, Figure S2). This resulted in a significant delay as a result of salinity in mean flowering time in the early group of 3 days in 2005–2006 and –1 day in 2007–2008, while the mean delay in flowering time of the late group was 5 days in 2005–2006 and 4 days in 2007–2008 (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 2005–2006 in the late group. In 2005–2006, 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 2007–2008, the genotype-by-treatment interaction was significant for all parameters except pod number in the early group. By contrast, the

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 2005–2006 and 2007–2008

	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
2005–2006												
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
2007–2008												
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

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 2007–2008 (Figure S3). Under non-saline conditions, seed yield in the late group varied significantly among genotypes in 2005–2006, whereas seed yield did not vary in 2007–2008. 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 a significant genotypic effect across both years and within each phenology group (Table 1).

In the early group, seed yield decreased by 24 and 52% under saline conditions in 2005–2006 and 2007–2008, respectively. In the late group, seed yield did not decrease in 2005–2006, but the higher NaCl level used in 2007–2008 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 a 7% decrease in 2005–2006 in the late group. Only in 2007–2008 was the decrease in the late group 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 2005–2006 and 2007–2008, respectively, and by 17 and 24% in the late group. Seed number decreased by 5 and 40% in 2005–2006 and

2007–2008, respectively, in the early group. Surprisingly, in the late group the saline treatment increased seed number by 19% in 2005–2006, but the more severe treatment in 2007–2008 decreased it by 18%.

The heritability of seed yield in the saline treatment was high in both phenology groups in 2005–2006, close to 0.78, although it decreased to about 0.60 with the higher salinity used in 2007–2008. 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 2007–2008 while heritability for seed number was up to 0.86 in 2005–2006 and heritability changed little in 2007–2008 (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 2 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 2005–2006 and 2007–2008, respectively; in the late group, $R^2 = 0.12$ and 0.00 in 2005–2006 and 2007–2008, 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 account 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 2005–2006 and 2007–2008), but the yield ratio was poorly related to the seed yield under saline conditions, except in one case (2005–2006: $R^2 = 0.21$ and 0.55 in the early and late group; 2007–2008: $R^2 = 0.06$ 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 2005–2006 and 2007–2008, respectively, polynomial fit not shown), there was a strong clustering of entries by phenology group, especially in 2007–2008 when the treatment was more severe (100 mM NaCl) (Figure S3). 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 2005–2006 and 2007–2008, respectively), with higher seed yield in later entries. Within the late group, there was no relationship between seed yield and flowering time (Figure S3).

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 2005–2006 and 2007–2008, 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 2005–2006 ($R^2 = 0.27$) and not significant in the higher salt treatment in 2007–2008 ($R^2 = 0.01$) (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 2007–2008 ($R^2 = 0.16$): early group, $R^2 = 0.53$ in 2005–2006; late group, $R^2 = 0.47$ and 0.46 in 2005–2006 and 2007–2008, respectively. Figure 2 also separates seed number under salinity between the early and late

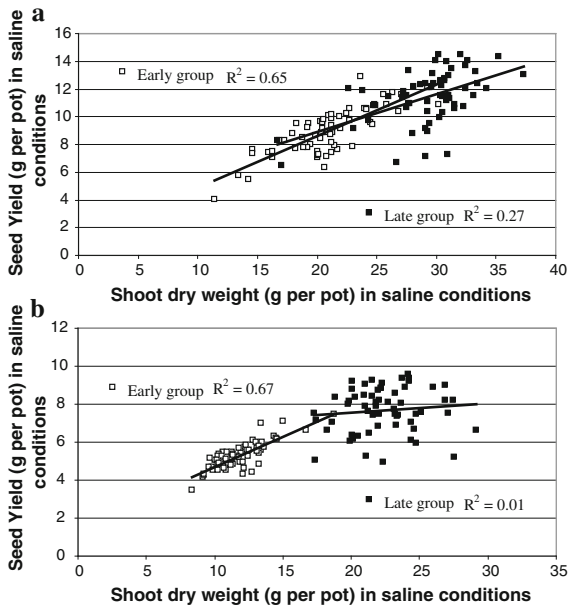

Fig. 1 Relationship between seed yield and shoot dry weight in saline conditions in 2005–2006 (**a**) and 2007–2008 (**b**) within two phenology groups: early group (flowering within 38 days after sowing in saline conditions, *open symbols*) and late group (flowering 41 days or more after sowing in saline conditions, *closed symbols*). Data are means of four replicate pots per genotype with four plants per replicate pot. *Lines* are fitted linear regressions

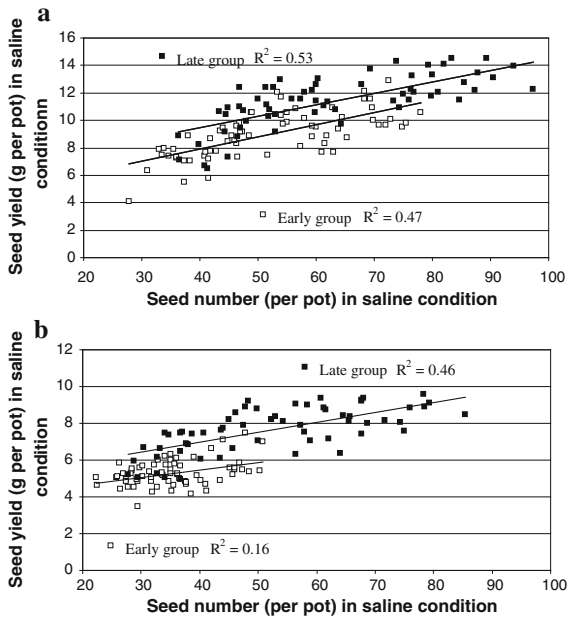

Fig. 2 Relationship between seed yield and seed number in saline conditions in 2005–2006 (**a**) and 2007–2008 (**b**) within two phenology groups: early group (flowering within 38 days after sowing in saline conditions, *open squares*) and late group (flowering 41 days or more after sowing in saline conditions, *solid squares*). Data are means of four replicate pots per genotype with four plants per replicate pot. *Lines* are fitted linear regressions

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 (Figure S4).

Linkage map and QTL analysis

Of the 216 markers tested, 135 markers were mapped on to eight linkage groups (LGs) spanning a distance of 310.2 cM, although 81 markers remained unmapped. Linkage groups were assigned to chromosomes based on the 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). The overall inter-marker distance was 2.3 cM (Fig. 3). QTL 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 2007–2008, explaining 13% of the variation, and one QTL for seed number under salinity was found on LG7 in 2007–2008, explaining 25% of the variation (Table 2). No QTL were detected for yield ratio among the early phenology group.

In the late phenology group, a QTL was found for seed yield under salinity on LG3 in 2007–2008, 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

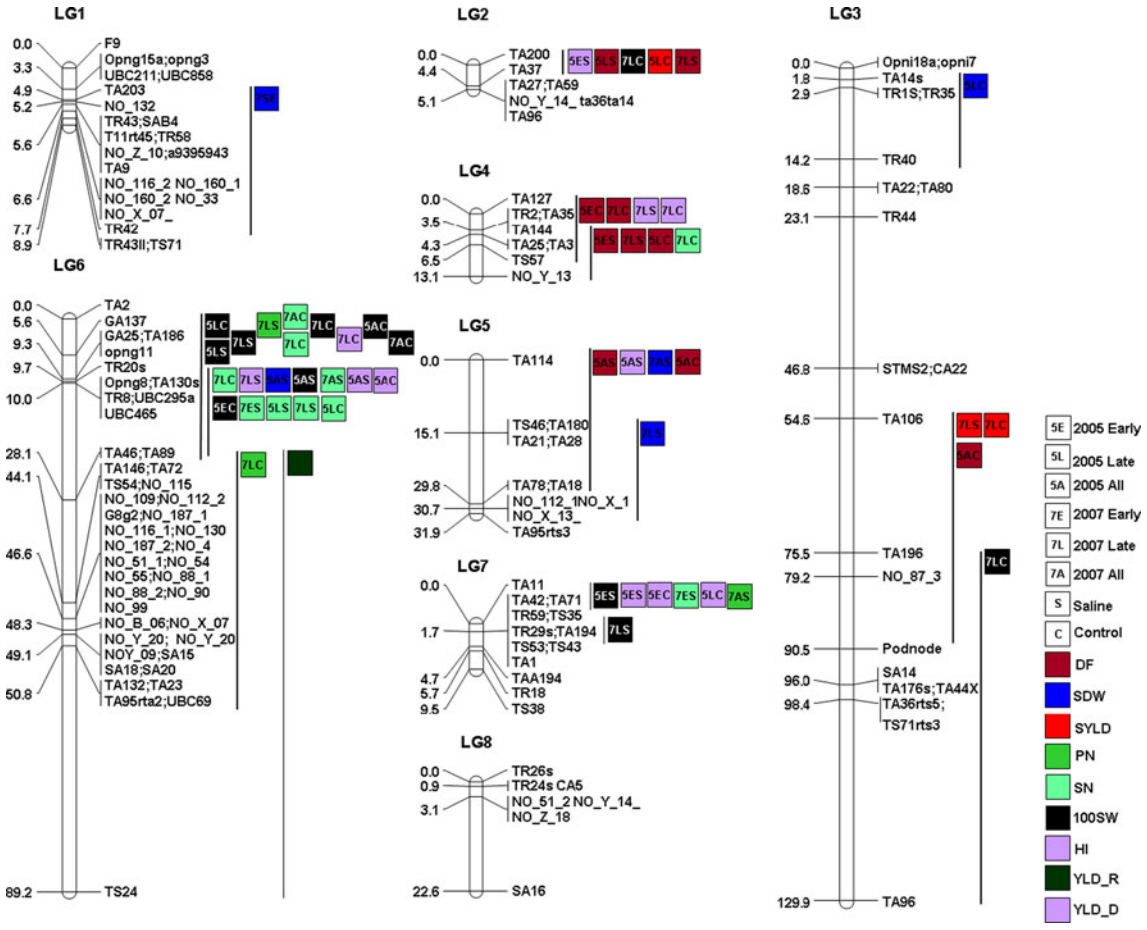

Fig. 3 Genetic linkage map of chickpea (ICCV 2 × JG 62) with 135 marker loci on eight linkage groups. Kosambi map distances are on *left-hand side*, genomic regions harboring QTL (*black bars*) and QTL for salinity-related traits (*colored squares*), as listed in Table 2, on *right-hand side* of linkage group for early phenology group (*E*), late phenology group (*L*), under both saline (*S*) and non-saline (*C*) conditions and two environments 2005–2006 (*5*) and 2007–2008 (*7*)

different genomic region, for shoot dry weight in 2007–2008 and for yield in 2005–2006 and 2007–2008 (Table 2; Figure S5). Among the surrogates for seed yield under salinity, a genomic region was identified on LG6 that contained QTL for pod number, seed number, 100-seed weight under salinity as well as non-saline (control) conditions in 2007–2008. The QTL for pod number under salinity in this genomic region explained as much as 37% of the phenotypic variation. Similarly, QTL for seed number and 100-seed weight were found under both saline and non-saline conditions during 2005–2006 in the same genomic region on LG6, where QTL for pod number, seed number, and 100-seed weight under saline and non-saline conditions in 2007–2008 were found (Table 2; Figures S6 and S7). One QTL for flowering time was also found consistently across treatment and year of experiment, located on LG4 (Figure S6). 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 2007–2008 and contributed a phenotypic variation of 34.6%.

When the phenotyping data were used for QTL analysis, disregarding the groups of phenology, no QTL for seed yield and yield ratio were found in any of the treatments for either of the 2 years. Nevertheless, a genomic region containing QTL for seed number and 100-seed weight under saline conditions in both 2005–2006 and 2007–2008 was found on LG6. The same genomic region also contained QTL

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 rato(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 2005–2006 and 2007–2008

Trait	Early genotypes				Late genotypes				All genotypes			
	LG	Marker interval	LOD	PV (%)	LG	Marker interval	LOD	PV (%)	LG	Marker interval	LOD	PV (%)
2005–2006												
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
HI	2	TA200–TA37	2.5	11.9	–	–	–	–	5	TA114–NO_X_1	3.1	11.5
Control												
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	–	–	–	–
2007–2008												
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	–	–	–	–

Table 2 continued

Trait	Early genotypes				Late genotypes				All genotypes			
	LG	Marker interval	LOD	PV (%)	LG	Marker interval	LOD	PV (%)	LG	Marker interval	LOD	PV (%)
2005												
YLD_R		–	–	–	–	–	–	–	–	–	–	–
YLD_D–	–	–	–	–	–	–	–	–	–	–	–	–
2007												
YLD_R–	–	–	–		6	TA46–TS24	5.4	34.6	–	–	–	–
YLD_D–	–	–	–	–	–	–	–	–	–	–	–	–

for seed number and 100-seed weight under non-saline conditions in both years (Figure S7).

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 QTL 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 under saline conditions/seed yield under non-saline conditions). This ratio was in fact poorly related to the seed yield under saline conditions and this reflects the fact 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 for 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 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 underway to better understand the process(es) affected during reproduction.

An interesting difference from previous work (Vadez et al. 2007) was the significant relationship between seed yield and shoot dry weight in the 'early' group of entries. Serraj et al. (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 2007–2008, 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 2005–2006 and 0.04 in 2007–2008). 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 reduced seed numbers in the early entries. Seed number increased under salinity in 2005–2006 in the late group, and was the trait best correlated to seed yield ($R^2 = 0.53$), whereas shoot biomass was decreased by 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 that 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. absissic acid (ABA)), as increased ABA has previously been reported to delay flowering (Achard et al. 2006).

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 QTL for salinity tolerance, and

most are QTL for traits such as sodium exclusion in rice (Ren et al. 2005) or growth (Takehisa et al. 2004). Fewer studies again have identified QTL 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, QTL 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 small percentage of the variation. Combined QTL analysis of the entire RIL population did not reveal any QTL, highlighting the importance of first elucidating the role of phenology in the genotypic response to salt stress. QTL 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 QTL 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 2 years and treatments. These QTL 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 traits 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 (2005–2006) or of a significant (2007–2008) genotype-by-treatment interaction for these traits in the late group (Supplementary Table 1). Similarly, a genomic region on LG4 harboring QTL 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 QTL 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, QTL 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 the 2007–2008 environment, although, as expected, this QTL had no relationship with the cluster of QTL on LG6 for salinity tolerance surrogates such as seed number or pod number.

Conclusion

This is the first report on QTL 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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References

Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, Moritz T, Van Der Straeten D, Peng J, Harberd NP (2006) Integration of plant responses to environmentally activated phytohormonal signals. Science 311:91–94

Anuradha C, Gaur PM, Pande S, Gali KK, Ganesh M, Kumar J, Varshney RK (2011) Mapping QTL for resistance to botrytis grey mould in chickpea. Euphytica. doi: 10.1007/s10681-011-0394-1 (in press)

Cho S, Jagdish Kumar J, Shultz JL, Anupama K, Tefera F, Anupama K, Tefera F, Muehlbauer FJ (2002) Mapping genes for double podding and other morphological traits in chickpea. Euphytica 128:285–292

Datta KS, Dayal J, Goswami CL (1987) Effect of salinity on growth and yield attributes of chickpea (*Cicer arietinum* L). Ann Biol 3:47–53

Dhingra HR, Varghese TM (1993) Flowering and male reproductive functions of chickpea (*Cicer arietinum* L) genotypes as affected by salinity. Biol Plant 35:447–452

Ellis RP, Forster BP, Gordon DC, Handley LL, Keith RP, Lawrence P, Meyer R, Powell W, Robinson D, Scrimgeour CM, Young G, Thomas WTB (2002) Phenotype/genotype associations for yield and salt tolerance in a barley mapping population segregating for two dwarfing genes. J Exp Bot 53:1163–1176

Flowers TJ, Gaur PM, Gowda CLL, Krishnamurthy L, Samineni S, Siddique KMH, Turner NC, Vadez V, Varshney RK, Colmer TD (2010) Salt sensitivity in chickpea. Plant Cell Environ 33:490–509

Gregorio GB, Senadhira D, Mendoza RD, Manigbas NL, Roxas JP, Guerta CQ (2002) Progress in breeding for

salinity tolerance and associated abiotic stresses in rice. Field Crops Res 76:91–101

Katerji N, van Hoorn JW, Hamdy A, Mastrorilli M, Oweis T, Malhotra RS (2001) Response to soil salinity of two chickpea varieties differing in drought tolerance. Agric Water Manag 50:83–96

Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eugen 12:172–175

Krishnamurthy L, Johansen C, Sethi SC (1999) Investigation of factors determining genotypic differences in seed yield of non-irrigated and irrigated chickpeas using a physiological model of yield determination. J Agron Crop Sci 183: 9–17

Krishnamurthy L, Turner NC, Gaur PM, Upadhyaya HD, Varshney RK, Siddique KHM, Vadez V (2011) Consistent variation across years in salinity resistance in a diverse range of chickpea (*Cicer arietinum* L.) genotypes. J Agron Crop Sci. doi: 10.1111/j.1439-037X.2010.00456.x

Lander ES, Green J (1987) Construction of multilocus genetic linkage maps in human. P Nat Acade Sci USA 84: 2363–2367

Lauter DJ, Munns DN (1986) Salt resistance of chickpea genotypes in solutions salinized with NaCl or Na_2SO_4. Plant Soil 95:271–279

Lee GJ, Boerma HR, Villagarcia MR, Zhou X, Carter TE Jr, Li Z, Gibbs MO (2004) A major QTL conditioning salt tolerance in S-100 soybean and descendent cultivars. T Appl Genet 109:1610–1619

Leport L, Turner NC, French RJ, Tennant D, Thomson BD, Siddique KHM (1998) Water relations, gas exchange and growth of cool-season grain legumes in a Mediterranean-type environment. Eur J Agron 9:295–303

Leport L, Turner NC, French RJ, Barr MD, Duda R, Davies SL, Tennant D, Siddique KHM (1999) Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment. Eur J Agron 11:279–291

Leport L, Turner NC, Davies SL, Siddique KHM (2006) Variation in pod production and abortion among chickpea cultivars under terminal drought. Eur J Agron 24:236–246

Mamo T, Richter C, Heiligtag B (1996) Salinity effects on the growth and ion contents of some chickpea (*Cicer arietinum* L) and lentil (*Lens culinaris medic*) varieties. J Agron Crop Sci 176:235–247

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Ann Rev Plant Biol 59:651–681

Nayak SN, Zhu H, Varghese N, Choi HK, Datta S, Horres R, Jüngling R, Singh J, Kavi Kishor PB, Kahl G, Winter P, Cook DR, Varshney RK (2010) Integration of novel SSR and gene-based marker loci in the chickpea genetic map and establishment of new anchor points with *Medicago truncatula* genome. Theor Appl Genet 120:1415–1441

Nayyar H, Kaur S, Singh KJ, Dhir KK, Bains T (2005) Water stress-induced injury to reproductive phase in chickpea: Evaluation of stress sensitivity in wild and cultivated species in relation to abscisic acid and polyamines. J Agron Crop Sci 191:450–457

Quarrie SA, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusić D, Waterman E, Weyen J, Schondelmaier J, Habash DZ, Farmer P, Saker L, Clarkson DT, Abugalieva A, Yessimbekova M, Turuspekov Y, Abugalieva S, Tuberosa R, Sanguineti MC, Hollington PA, Aragués R, Royo A, Dodig D (2005) A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring × SQ1 and its use to compare QTLs for grain yield across a range of environments. T Appl Genet 110:865–880

Radhika P, Gowda SJM, Kadoo NY, Mhase LB, Jamadagni BM, Sainani MN, Chandra S, Gupta VS (2007) Development of an integrated intraspecific map of chickpea (*Cicer arietinum* L.) using two recombinant inbred line populations. Theor Appl Genet 115:209–216

Rangasamy P (2006) World salinization with emphasis on Australia. J Exp Bot 57:1017–1023

Ren ZH, Gao JP, Li LG, Cai XL, Huang W, Chao DY, Zhu MZ, Wang ZY, Luan S, Lin HX (2005) A rice quantitative trait locus for salt tolerance encodes a sodium transporter. Nature Genet 37:1141–1146

Richards RA (1983) Should selection for yield in saline regions be made on saline or non-saline soils. Euphytica 32: 431–438

Samineni S, Siddique KHM, Gaur PM, Colmer TD (2011) Salt sensitivity of the vegetative and reproductive stages in chickpea (*Cicer arietinum* L.): podding is a particularly sensitive stage. Environ Exp Bot 71:260–268

Saxena NP (1984) Chickpea. In: Goldworthy PR, Fisher NM (eds) The physiology of tropical field crops. Wiley, New York, pp 419–452

Serraj R, Krishnamurthy L, Upadhyaya HD (2004) Screening chickpea mini-core germplasm for tolerance to soil salinity. Int Chickpea Newsl 11:29–32

Takehisa H, Shimodate T, Fukuta Y, Ueda T, Yano M, Yamaya T, Kameya T, Sato T (2004) Identification of quantitative trait loci for plant growth of rice in paddy field flooded with salt water. Field Crops Res 89:85–95

Vadez V, Krishnamurthy L, Serraj R, Gaur PM, Upadhyaya HD, Hoisington DA, Varshney RK, Turner NC, Siddique KHM (2007) Large variation in salinity tolerance in chickpea is explained by differences in sensitivity at the reproductive stage. Field Crops Res 104:123–129

Varshney RK, Hoisington DA, Upadhyaya HD, Gaur PM, Nigam SN, Saxena K, Vadez V, Sethy NK, Bhatia S, Aruna R, Gowda MVC, Singh NK (2007) Molecular genetics and breeding of grain legume crops for the semi-arid tropics. In: Varshney RK, Tuberosa R (eds) Genomics assisted crop improvement, vol II: genomics applications in crops. Springer, Dordrecht, pp 207–242

Varshney RK, Hoisington DA, Nayak SN, Graner A (2009) Molecular plant breeding: methodology and achievements. In: Somers D, Langridge P, Gustafson PJ (eds) Methods in molecular biology: plant genomics. The Humana Press, Totowa, pp 283–304

Wang S, Basten CJ, Zeng ZB (2010) Windows QTL cartographer 2.51. Department of Statistics, North Carolina State University, Raleigh, NC. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm

Winter P, Benko-Iseppon AM, Hüttel B, Ratnaparkhe M, Tullu A, Sonnante G, PfaV T, Tekeoglu M, Santra D, Sant VJ, Rajesh PN, Kahl G, Muehlbauer FJ (2000) A linkage map of the chickpea (*Cicer arietinum* L.) genome based on the recombinant inbred lines from a *C. arietinum* × *C. reticulatum* cross: localization of resistance genes for *Fusarium* races 4 and 5. T Appl Genet 101:155–1163