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Estimation of genetic components of variation for salt tolerance in chickpea using the generation mean analysis

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Abstract Chickpea (*Cicer arietinum* L.) is known to be salt-sensitive and in many regions of the world its yields are restricted by salinity. Recent identification of large variation in chickpea yield under salinity, if genetically controlled, offers an opportunity to develop cultivars with improved salt tolerance. Two chickpea land races, ICC 6263 (salt sensitive) and ICC 1431 (salt tolerant), were inter-crossed to study gene action involved in different agronomic traits under saline and control conditions. The generation mean analysis in six populations, viz. $P_1, P_2, F_1, F_2, BC_1P_1$ and BC_1P_2, revealed significant gene interactions for days to flowering, days to maturity, and stem Na and K concentrations in control and saline treatments, as well as for 100-seed weight under salinity. Seed yield, pods per plant, seeds per plant, and stem Cl concentration were controlled by additive effects under saline conditions. Broad-sense heritability values (>0.5) for most traits were generally higher in saline than in control conditions, whereas the narrow-sense heritability values for yield traits, and stem Na and K concentrations, were lower in saline than control conditions. The influence of the sensitive parent was higher on the expression of different traits; the additive and dominant genes acted in opposite directions which led to lower heritability estimates in early generations. These results indicate that selection for yield under salinity would be more effective in later filial generations after gene fixation.

Keywords Chickpea · *Cicer arietinum* · Generation mean analysis · Genetics · Salinity tolerance · Tissue sodium and chloride

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Introduction

Salinity affects soils in more than 100 countries and salinization appears to be most prevalent in arid and semi-arid regions (Rengasamy 2006). Chickpea (*Cicer arietinum* L.), a self-pollinated diploid, is globally the third most important food legume in terms of production after common bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.) (FAOSTAT 2011). Chickpea is grown in areas of low to moderate rainfall on soils where capillary rise often transports

salts towards the surface. Many studies have indicated that chickpea is sensitive to salinity (Flowers et al. 2010; Lauter and Munns 1987). Thus, the development of cultivars with enhanced salt tolerance is a pressing matter for yields to remain stable, and particularly in arid and semi-arid regions.

Breeding for salt tolerance has received relatively little attention in grain legumes. Genetic control of different traits under salinity has been studied in only a few major grain legumes, such as soybean, pigeonpea and chickpea. In soybean, average leaf scorching was used as a measure of salt tolerance and this trait showed monogenic inheritance (Lee et al. 2009). In pigeonpea, only additive gene effects were significant for seed yield in saline conditions (Ashraf 1998). In chickpea, dominant effects mostly controlled seed yield in saline conditions, with minor contributions from additive effects (Ashraf and Waheed 1998). In other crops such as rice, wheat, barley and tomato, there are many reports on inheritance of salinity tolerance and various sources of tolerance have been identified for breeding programs (Akbar et al. 1986; Foolad 1997; Koval and Rigin 1993; Munns et al. 2003; Colmer et al. 2005). More emphasis on improving salt tolerance in grain legumes, especially chickpea, would benefit people in arid and semi-arid regions of the world where this crop is a major source of protein and soils are prone to salinization.

Ion accumulation (usually Na) in vegetative tissues has been reported as an important trait influencing salt tolerance in a range of crop species (Yan et al. 1992; Cramer et al. 1994; Foolad 1997; Munns et al. 2003; Munns and Tester 2008). Legumes appear to be more sensitive to salinity than other crop plants. In chickpea exposed to saline conditions, toxic accumulation of Na and Cl has been reported in different plant parts at different growth stages (Murumkar and Chavan 1986; Lauter and Munns 1987; Mamo et al. 1996; Samineni et al. 2011). In a large-scale salinity screening in chickpea, however, Na concentration in vegetative shoots had no relationship with the biomass or the final seed yield (Vadez et al. 2007). For other grain legumes such as soybean, salt tolerance was associated with exclusion of Na by roots, preventing accumulation of toxic concentrations in stems and leaves (Luo et al. 2005). No report is available on the inheritance of tissue Na or Cl concentrations in chickpea, nor in other grain legume species, under salt stress.

Genetic variation is a pre-requisite for breeding through selection. In chickpea, early research found low levels of diversity for salt tolerance (Lauter and Munns 1986; Johansen et al. 1990; Dua 1992) which was further complicated by variable tolerance levels at different growth stages (Ashraf and Waheed 1993). A recent study, however, identified chickpea genotypes with large variation in yield at 80 mM NaCl in soil (Vadez et al. 2007). The present study used genotypes contrasting for salinity tolerance selected from Vadez et al. (2007) to study the components of genetic variation for different traits under salinity using generation mean analysis (GMA) (Cavalli 1952; Mather 1949). Traits studied included yield components, above-ground biomass, stem ion concentrations, and phenological traits, in generations P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2 of a cross between sensitive (ICC 6263) and tolerant (ICC 1431) chickpea, when grown in control or salinized soil (80 mM NaCl) in pots in a semi-field situation.

Materials and methods

Experimental procedure

The experimental material consisted of six populations (parents P_1 and P_2, F_1 and F_2, and first backcross generations to each parent, BC_1P_1 and BC_1P_2) developed from the cross ICC 6263 (P_1) × ICC 1431 (P_2). The two parental lines were developed as pure lines by selfing a single plant in each accession ICC 6263 and ICC 1431, which were selected based on their 2-year (2003/2004 and 2004/2005) yield performance under saline (80 mM NaCl) soil conditions (Vadez et al. 2007) at ICRISAT, India (latitude 17.53°N, longitude 78.27°E; altitude 545 m above mean sea level). ICC 6263 is a kabuli type chickpea (white flower) with poor yield in saline conditions (64% less than control) whereas ICC 1431 is a desi type (pink flower) with a considerably lower yield reduction (26% less than control) in saline soil (80 mM NaCl; Vadez et al. 2007). These parents have similar phenologies (days to flowering at ICRISAT: ICC 6263 = 61; ICC 1431 = 59) and 100-seed weight (ICC 6263 = 23 g; ICC 1431 = 21 g). Some F_1 hybrids were self-pollinated to produce the F_2 population, while others were used to generate backcross populations. The progeny

derived from backcrossing F_1 to parent P_1 was designated BC_1P_1 and that to parent P_2 as BC_1P_2. The hybrids had pink flowers, confirming their hybridity (pink is dominant over white).

The experiment was conducted with a movable rainout shelter at ICRISAT; the shelter only covered the experiment during the few rainfall events. The experiment was conducted during spring 2008/2009, and the average minimum and maximum temperatures were 11°C and 35°C, respectively.

Black soil (Vertisol, top 20 cm layer) was taken from the ICRISAT farm for the experiment (Vadez et al. 2007). The soil had been dried under the sun, sieved through 4 mm mesh, and steam sterilized for two cycles (4 h each cycle), prior to use. Plastic pots (20 cm diameter) were each filled with 4.5 kg of soil and buried in the ground to reduce the heat effect on the roots. Seeds were treated with a fungicide mixture (thirum + captan) before sowing. A single plant was grown in each pot.

All six populations were grown in a randomized block design with two treatments (control and 80 mM NaCl) and three replicates. The sample sizes (i.e. number of plants analyzed; single plant per replicate pot) in each replicate block were: 12 plants each for P_1 and P_2; 8 plants for F_1; 120 plants for F_2; and 30 plants each for BC_1P_1 and BC_1P_2. Pots were completely randomized within each of the three blocks.

Salt was applied through the irrigation water at the time of sowing. Initially, 1,350 ml of water (equal to the pot 'field capacity') was applied with or without NaCl. Further irrigations were on alternate days with such volumes applied that pots were never under or over irrigated (i.e. to avoid waterlogging or water stress based on visual observations and experience in running these experiments, e.g. see Vadez et al. 2007). Preventive measures were taken to raise a healthy crop and no diseases or pests were observed.

The traits assessed were days to flowering, days to maturity (leaves had all turned brown/yellow and no further flowers formed), plant biomass (dry weight of vegetative and reproductive structures at harvest, after oven-drying at 65°C), harvest index, number of pods per plant, % empty pods (empty pods relative to the total number of pods on a plant), number of seeds per plant, weight (g) of 100-seeds and seed yield per plant (g).

Ion analysis

At maturity, stems (shoots devoid of leaves) were oven-dried at 65°C for 2 days and ground to pass through a 1 mm sieve. The ground stem samples were treated with 0.5 M HNO_3 in plastic tubes placed on a horizontal shaker for 48 h. The acid extract was diluted with milli-Q water, and Na and K were analyzed using a flame photometer (Model PFP7, Jenway, Essex, UK) and Cl was measured with a chloridometer (Chloridometer 50 cl, SLAMED Laboratory Instruments, Germany). A plant reference material, broccoli (*Brassica oleracea*) was used as a standard check for these analyses. The recovery rates of Na, K and Cl were 94, 98 and 95%, respectively (data not adjusted).

Calculations

Variance components (additive, dominance and environment) were estimated as described by Mather and Jinks (1971) using the following equations:

$$\text{Environmental variance or error } \left[\sigma^2 e\right] = \tfrac{1}{4}\left[\sigma^2 P_1 + \sigma^2 P_2 + \left(2\sigma^2 F_1\right)\right]$$

$$\text{Genotypic variance in } F_2 \left[\sigma^2 G(F_2)\right] = \sigma^2 F_2 - \sigma^2 e$$

$$\text{Additive variance in } F_2 \left[\sigma^2 A(F_2)\right] = \left(2\sigma^2 F_2\right) - \left(\sigma^2 BC_1P_1 + \sigma^2 BC_1P_2\right)$$

$$\text{Variance of dominance in } F_2 \left[\sigma^2 D(F_2)\right] = \sigma^2 G(F_2) - \sigma^2 A(F_2)$$

$$\text{Broad-sense heritability } h^2(a) = 100\left[\sigma^2 G(F_2)/\sigma^2(F_2)\right]$$

$$\text{Narrow-sense heritability } h^2(e) = 100\left[\sigma^2 A(F_2)/\sigma^2(F_2)\right]$$

The generation mean analysis of the six populations (P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2) and associated scaling tests (Cavalli 1952; Mather 1949) were performed based on the assumption that populations have non-homogeneous variances (Mather and Jinks 1971). A statistical explanation supports the theory that the variance of the populations will not be homogeneous (Beaver and Mosjidis 1988). The variation in the parental lines and their F_1 is environmental, whereas variation in later generations

has both genetic and environmental components (Mather and Jinks 1971). The validity of the additive–dominance models for the scaling test and the joint scaling test were examined using WINDOSTAT 8.5 software (Indostat services, Hyderabad, India, http://www.windostat.org/index.htm).

The gene effects were estimated from the joint scaling test as proposed by Mather and Jinks (1982) using WINDOSTAT. This program first tries to fit three-parameter models, deletes those with t values <2.0, then tests the model significance by a weighted chi-square (χ^2) test. If significant, the program tries to fit a six-parameter model ([m] = mid parental values; [d] = additive effects; [h] = dominance effects; [i] = additive by additive; [j] = additive by dominance; [l] = dominance by dominance) with a step-down for non-significant parameters. If all parameters are statistically significant, the program computes a weighted χ^2 test for the joint scaling test. The weight of each population was calculated as the inverse of variance of the generation mean. The degree of the dominance ratio was measured using $[H/D]^{1/2}$, where H is dominance variance and D is additive variance.

Results

Effect of salt stress on flowering, maturity and biomass

Salinity delayed days to flowering, but did not significantly affect days to maturity, in any generation (Table 1). The longest delay in flowering was 10 days in BC_1P_2 and the shortest was 2 days in F_1. The variation in flowering was due to the direct effect of salinity since the confounding effect of flowering time had been removed by selecting parents with similar phenology (Table 1).

Days to flowering and days to maturity had significant χ^2 estimates for the three-parameter model under control and saline conditions. The additive dominance model did not explain genetic variation for days to flowering and days to maturity (Table 2). For days to flowering, dominance effects were highly significant along with epistatic interactions. Under saline conditions, flowering was influenced primarily by dominance effects, whereas under non-saline conditions the influences were additive and additive by dominance [j] interactions. The dominant and dominant by dominant [l] effects were significant in opposite directions which suggest a duplicate interaction of genes controlling days to flowering and days to maturity (Table 2). In control plants, both days to flowering and days to maturity had complementary gene effects. This indicates different types of gene effects control these traits under control and saline conditions. The negative effect of salt on the flowering time was mainly controlled by a dominant by dominant effect [l] and on maturity by an additive by dominant effect [j].

Salinity decreased shoot biomass by 26% in both the sensitive and tolerant parent. The decrease in shoot biomass was greater in both backcross populations; 30% when backcrossed to ICC 1431 (tolerant) and 39% when backcrossed to ICC 6263 (sensitive) (Table 1). In controls, biomass was mainly controlled by epistatic gene effects such as additive by additive [i] and additive by dominance [j] apart from mean effects, whereas under saline conditions no interactions were observed and only mean effects were significant (Table 2). In summary, parental genotypes did not differ significantly for biomass at 80 mM NaCl.

Effect of salt stress on yield components

Under saline treatment, seed yield of sensitive (ICC 6263) and tolerant (ICC 1431) parents decreased by 76 and 46%, respectively (Table 1). The yield decrease in F_1 was intermediate but closer to the sensitive parent. Among F_2 segregants, more plants produced fewer pods and seeds which lowered seed yields. The yield reduction in BC_1P_1 and BC_1P_2 were similar to the recurrent parents. Salinity reduced the 100-seed weight and this reduction was higher in sensitive parent (36%) than for the tolerant parent (26%). The reductions in 100-seed weight were even more pronounced in F_1, F_2 and BC_1 with the sensitive parent (43–58%).

In both control and salt treatments, variation among the means of different generations for yield traits was sufficiently explained by a simple additive–dominance model (Table 2). The best estimation of the additive and dominance effects came from the three-parameter model (m, d and h) because these effects were unbiased due to the absence of interactions (Hayman 1958). All yield characteristics, except the 100-seed weight in saline conditions, were

Table 1 Comparisons of means (±standard errors) for various characters in six populations of cross ICC 6263 × ICC 1431 grown in control and saline (80 mM NaCl) treatments (each value is an average of three replications)

Character	Populations					
	P_1	P_2	F_1	F_2	BC_1P_1	BC_1P_2
Days to flowering						
C	57.39 ± 0.34	57.89 ± 0.25	55.79 ± 0.49	59.99 ± 0.32	58.99 ± 0.56	57.81 ± 0.44
S	61.06 ± 0.45	64.00 ± 0.32	57.58 ± 0.46	66.41 ± 0.36	66.47 ± 0.63	66.21 ± 0.25
%	+6.39*	+10.55*	+3.59*	+10.70*	+12.68*	+14.53*
Days to maturity						
C	98.78 ± 0.25	98.83 ± 0.41	95.67 ± 0.47	98.62 ± 0.31	98.82 ± 0.46	98.63 ± 0.33
S	100.66 ± 0.39	102.50 ± 0.30	97.83 ± 0.39	99.90 ± 0.39	101.71 ± 0.62	99.20 ± 0.37
%	+1.91 ns	+3.71 ns	+2.26 ns	+1.30 ns	+2.92 ns	+0.58 ns
Shoot biomass (g)						
C	6.48 ± 0.53	8.32 ± 1.17	9.23 ± 1.27	9.17 ± 0.55	9.38 ± 0.57	7.52 ± 0.33
S	4.79 ± 0.59	6.12 ± 0.82	6.26 ± 0.79	5.36 ± 0.36	5.74 ± 0.76	5.24 ± 0.58
%	−26.12*	−26.44*	−32.18*	−41.55*	−38.81*	−30.32*
Harvest index						
C	0.44 ± 0.02	0.47 ± 0.01	0.48 ± 0.04	0.43 ± 0.01	0.43 ± 0.01	0.46 ± 0.01
S	0.20 ± 0.01	0.35 ± 0.03	0.26 ± 0.03	0.14 ± 0.01	0.13 ± 0.01	0.23 ± 0.02
%	−55.55*	−27.08*	−45.83*	−67.44*	−69.77*	−50.00*
Pods per plant						
C	13.89 ± 0.89	17.67 ± 3.03	16.71 ± 2.56	14.98 ± 1.06	14.40 ± 1.13	13.60 ± 0.76
S	4.77 ± 0.45	12.19 ± 2.06	9.33 ± 1.72	6.32 ± 0.67	6.52 ± 1.53	8.71 ± 1.32
%	−65.60*	−31.01*	−44.16*	−57.81*	−54.72*	−35.95*
Seeds per plant						
C	14.00 ± 0.95	20.86 ± 3.76	20.62 ± 3.08	19.74 ± 1.48	18.10 ± 1.40	17.37 ± 1.12
S	5.41 ± 0.47	14.05 ± 2.25	11.12 ± 2.09	7.82 ± 0.87	7.78 ± 1.79	10.64 ± 1.97
%	−61.90*	−32.65*	−46.10*	−60.39*	−57.01*	−38.75*
Empty pods						
C	0.55 ± 0.21	1.17 ± 0.28	1.25 ± 0.46	1.02 ± 0.09	1.02 ± 0.19	1.06 ± 0.17
S	1.22 ± 0.33	1.75 ± 0.48	1.50 ± 0.43	1.21 ± 0.10	0.98 ± 0.16	0.97 ± 0.16
%	+121.81*	+49.57*	+20.00*	+18.63*	−3.92 ns	−8.49*
Seed yield (g)						
C	3.58 ± 0.25	4.08 ± 0.71	4.57 ± 0.70	3.92 ± 0.26	4.09 ± 0.31	3.49 ± 0.19
S	0.85 ± 0.09	2.22 ± 0.42	1.57 ± 0.32	1.15 ± 0.18	1.21 ± 0.34	1.67 ± 0.34
%	−76.26*	−45.59*	−65.65*	−70.66*	−70.41*	−52.15*
100 Seed weight (g)						
C	23.68 ± 0.58	20.03 ± 0.41	19.59 ± 1.78	20.71 ± 0.41	22.40 ± 070	20.82 ± 0.59
S	15.04 ± 0.70	14.78 ± 0.52	11.07 ± 1.35	9.19 ± 0.49	9.43 ± 0.97	12.49 ± 0.88
%	−36.48*	−26.21*	−43.49*	−55.63*	−57.90*	−40.00*
Na in stems (μmol g^{-1} dry mass)						
C	45.18 ± 1.56	44.38 ± 1.95	45.25 ± 2.11	47.25 ± 0.99	36.46 ± 1.05	43.29 ± 1.69
S	224.00 ± 73.56	305.22 ± 11.77	290.63 ± 74. 95	241.06 ± 7.93	222.689 ± 14.59	270.55 ± 16.99
%	+4.96 times	+6.88 times	+6.42 times	+5.10 times	+6.11 times	+6.25 times
Cl in stems (μmol g^{-1} dry mass)						
C	343.07 ± 10.78	466.15 ± 15.29	350.41 ± 14.45	381.22 ± 5.62	341.51 ± 10.15	412.39 ± 10.66

Table 1 continued

Character	Populations					
	P_1	P_2	F_1	F_2	BC_1P_1	BC_1P_2
S	1060.37 ± 32.50	1668.11 ± 51.0	1387.44 ± 40.89	1411.02 ± 24.06	1199.72 ± 39.49	1473.96 ± 40.32
%	+3.10 times	+3.58 times	+3.96 times	+3.71 times	+3.81 times	+3.57 times
K in stems (μmol g^{-1} dry mass)						
C	478.70 ± 22.24	503.52 ± 25.78	347.41 ± 11.56	512.16 ± 9.89	483.72 ± 16.17	511.17 ± 15.22
S	552.55 ± 17.72	525.93 ± 21.92	474.96 ± 15.81	583.29 ± 8.61	576.09 ± 14.23	593.08 ± 13.88
%	+13.31*	+3.65 ns	+35.83*	+13.89*	+19.07*	+15.85*

% Percent decrease (−) or increase (+) due to salinity

* t Significance calculated at $P = 0.05$

explained adequately by the additive–dominance model (Table 2). Absence of interaction effects for yield traits in both control and saline conditions was supported by a non-significant weighted χ^2 analysis. Under non-saline conditions, the dominance effects were statistically significant for the number of pods and seeds per plant and seed yield, whereas in the saline treatment, these traits were controlled by the additive effects, as estimated from the three-parameter model. That is, under saline conditions additive gene effects were significant for pods per plant, seeds per plant and seed yield, whereas additive by additive and additive by dominance interactions were significant for 100-seed weight. The only significant mean effect under salinity was in controlling the number of empty pods. Salt sensitivity was dominant over tolerance for yield traits.

The number of empty pods increased significantly, by up to 20%, under saline treatment in both parents, F_1, and most F_2 segregants. In backcross populations, there was no significant increase in empty pods in the saline treatment, suggesting that variation in different generations was mainly due to an error component (80%) of total variation and not to genetic components (20%) (Table 3). The number of empty pods per plant was not correlated with yield, whereas the decline in numbers of pods and seeds per plant both contributed to the yield reduction.

Effect of salt stress on Na, Cl and K concentrations in stem tissues

Both parents in the control had similar Na concentrations in stems (~44 μmol g^{-1} dry weight). In the saline treatment, ICC 6263 (sensitive) and ICC 1431 (tolerant) accumulated 5.0 and 6.9 times more Na, respectively, than the controls (Table 1). Thus, the tolerant parent (ICC 1431) had 36% higher Na concentration in stems than the sensitive parent under saline treatment. In both F_1 hybrids, the Na concentration was close to the tolerant parent; many F_2 segregants had Na concentrations close to the sensitive parent; and backcross generations were closer to their recurrent parents. Under saline conditions, stem K concentration was similar in sensitive and tolerant parents, and have even increased in the F_1 population by 35% but increases were less than 20% in other populations (Table 1).

The additive–dominance model was rejected for stem Na and K concentrations as more than two scales were significant in the scaling test (data not shown). In the control, both Na and K concentrations in whole stems were controlled by dominant gene effects, and for Na also with all three types of epistasis but for K only additive by additive [i] and dominance by dominance [l]. Under saline conditions, both additive and dominant gene effects were significant, and very high dominance by dominance [l] type interactions were predominant in controlling stem Na concentration which shows the significant role of duplicate gene action. Similarly, K concentration was controlled by dominant and dominance by dominance [l] gene effects when grown in saline conditions (Table 2).

The stem Cl concentration was under significant additive and dominant effects in the non-saline controls but only additive effects when in saline soil along with mean effects; in both treatments epistatic gene effects were absent (Table 2). A difference in the stem Cl concentration was observed between the

Table 2 Estimates of gene effects (±SE of mean) in control (C) and salinity (S) for various traits in the cross ICC 6262 × ICC 1431 of chickpea using Mather and Jinks (1982) six-parameter model

Character	Gene effects							
	m	d	h	i	j	l	$\chi 2$	Epistasis
Days to flowering								
C[b]	64.02* (±1.95)	−0.25 (±0.21)	−7.87* (±5.11)	−6.38* (±1.94)	2.86* (±1.50)	−2.36 (±4.302)	4.10 ns	Complementary
S[b]	62.82* (±1.98)	−1.472* (±0.277)	19.60* (±5.04)	−0.29 (±2.900)	3.46 (±2.075)	−24.84* (±4.627)	0.02 ns	Duplicate
Days to maturity								
C[b]	98.39* (±1.72)	−0.03 (±0.24)	−3.65* (±4.33)	0.41 (±1.70)	0.43 (±1.24)	−6.38* (±2.81)	0.15 ns	Complementary
S[b]	99.37* (±2.13)	−0.917* (±0.25)	3.67* (±5.39)	2.22 (±2.19)	6.86* (±1.53)	−5.21* (±3.41)	2.83 ns	Duplicate
Shoot biomass								
C[b]	10.26* (±2.66)	−0.92 (±0.64)	−3.13 (±6.39)	−2.86* (±2.59)	5.55* (±1.84)	2.30 (±4.48)	2.09 ns	–
S[a]	5.19* (±0.44)	−0.35 (±0.44)	0.61 (±0.88)				3.27 ns	No epistasis
Harvest index								
C[a]	0.45* (±0.01)	−0.021* (±0.01)	0.03 (±0.02)				4.46 ns	No epistasis
S[b]	0.11* (±0.06)	−0.08* (±0.02)	−0.02 (±0.18)	0.17* (±0.06)	−0.05 (±0.06)	0.17* (±0.14)	0.217 ns	–
Pods per plant								
C[a]	11.35* (±1.08)	−1.01* (±0.95)	5.31* (±2.37)				7.42 ns	No epistasis
S[a]	7.23* (±0.86)	−2.50* (±0.86)	−0.36 (±1.8)				5.17 ns	No epistasis
Seeds per plant								
C[a]	13.51* (±1.33)	−2.02 (±1.22)	−8.66* (±2.87)				9.46 ns	No epistasis
S[a]	8.74* (±0.99)	−3.36* (±0.99)	−0.06 (±2.03)				3.48 ns	No epistasis
Empty pods								
C[a]	0.79* (±0.16)	−0.16 (±0.14)	0.54 (±0.34)				7.46 ns	No epistasis
S[a]	1.17* (±0.24)	−0.12 (±0.18)	−0.32 (±0.48)				4.73 ns	No epistasis
Seed yield								
C[a]	2.91* (±0.28)	0.05 (±0.24)	1.64* (±0.62)				6.37 ns	No epistasis
S[a]	1.36* (±0.18)	−0.52* (±0.18)	0.06 (±0.36)				2.63 ns	No epistasis
100 Seed weight								
C[a]	22.79* (±0.35)	2.64* (±0.33)	−3.26* (±0.87)				4.31 ns	No epistasis
S[b]	7.82* (±3.31)	0.13 (±0.43)	2.25 (±8.98)	7.09* (±3.28)	−6.38* (±2.76)	1.00 (±8.909)	1.85 ns	–
Na in stems								
C[b]	74.27* (±5.78)	0.40 (±1.25)	−79.06* (±15.02)	−29.49* (±5.64)	−14.45* (±4.71)	50.03* (±10.18)	0.10 ns	Duplicate
S[b]	242.36* (±55.38)	−40.61* (±7.28)	−53.48* (±150.47)	22.25 (±34.174)	−14.51 (±47.11)	101.75* (±61.552)	0.23 ns	Duplicate

Table 2 continued

Character	Gene effects							
	m	d	h	i	j	l	$\chi 2$	Epistasis
Cl in stems								
C[a]	406.27* (±8.09)	−64.48* (±7.83)	−54.05* (±15.87)				0.55 ns	No epistasis
S[a]	1408.58* (±26.10)	−236.82* (±26.24)	−17.69 (±48.87)				2.10 ns	No epistasis
K in stems								
C[b]	549.99* (±61.89)	−12.41 (±17.03)	51.28* (±163.59)	−58.88* (±59.49)	−36.07 (±55.96)	−253.86* (±105.59)	2.23 ns	Duplicate
S[b]	534.088* (±54.658)	13.315 (±14.093)	255.953* (±145.241)	5.152 (±52.810)	−60.607 (±48.732)	−315.078* (±96.563)	1.636 ns	Duplicate

ns non significant, *m* mean effect, *d* additive effect, *h* dominance effect, *i* additive × additive effect, *j* additive × dominance effect, *l* dominance × dominance effect

* $P < 0.05$

[a] Additive–dominance model is adequate

[b] Additive–dominance model is inadequate

parents, being 36% higher in the tolerant genotype in non-saline controls, and 44% higher in the tolerant parent in the saline treatment (Table 1). The mean values of F_1, F_2, BC_1P_1 and BC_1P_2 were intermediate between parents in control and salinity treatments. The proportional increase in stem Cl concentration under salinity in different populations varied from 3.1 times (P_1) to 4.0 times (F_1).

Data for the F_2 population grown in the saline treatment were analyzed for possible relationships amongst various traits (Fig. 1). Stem Na had a polynomial inverse 1st order relationship with seed yield ($R^2 = 0.18$, $P < 0.001$) and biomass ($R^2 = 0.15$, $P < 0.001$) and no relationship with the stem K concentration (Fig. 1a–c). Stem Cl had an inverse cubic relationship with seed yield and biomass, and a weak significant linear relationship with stem K (Fig. 1d–f). Stem K concentration had no relationship with biomass or seed yield under salinity (data not shown). Stem Na and Cl concentrations had a significant (cubic) relationship ($R^2 = 0.58$, $P < 0.001$) (Fig. 2b). A significant (linear) positive relationship ($R^2 = 0.75$, $P < 0.001$) was observed between the shoot biomass and seed yield, but this relationship was driven by less than 8% of plants obtained as transgressive segregants (Fig. 2a) with more than 90% of segregants within the 26% of maximum biomass observed (37 g) and 15% of maximum seed yield (20 g).

Heritability estimates

The estimates of additive, dominance, and environmental components of variance, broad-sense and narrow-sense heritabilities, degree of dominance and inbreeding depression for different traits in control and salinity treatments are presented in Table 3. Signs associated with the variances indicate the influence of sensitive (negative) and tolerant (positive) parents in the population (Table 3).

Days to flowering and days to maturity exhibited high broad-sense heritabilities (more than 85%) in both control and salinity treatments (Table 3). For 100-seed weight, low heritability was observed in the control due to the opposing action of additive and dominance variances, whereas predominance of dominance variance led to a higher heritability in the salinity treatment. Pods per plant, seeds per plant and seed yield all showed high narrow-sense

Table 3 Different components of genetic variances, degree of dominance and heritability estimates of various traits studied under control (C) and saline conditions (S) in chickpea

Character	Additive variance (D)	Dominance variance (H)	Environmental variance or error (E)	Degree of dominance	Broad-sense heritability h^2(a)	Narrow-sense heritability h^2(e)
Days to flowering						
C	29.32	4.54	4.16	5.61	0.89	76.85
S	48.67	−9.25	5.40	−3.65	0.88	108.53
Days to maturity						
C	41.98	−10.98	4.61	−1.47	0.87	117.89
S	59.97	−10.86	4.27	−2.00	0.92	112.35
Shoot biomass						
C	182.88	−104.63	32.84	1.91	0.70	164.61
S	10.02	18.65	17.35	−1.01	0.62	21.77
Harvest index						
C	0.02	−0.01	0.12	1.90	0.19	62.50
S	−0.03	0.03	0.02	0.48	0.02	−119.05
Pods per plant						
C	642.22	−409.47	171.62	−2.29	0.58	158.82
S	−51.50	132.61	77.09	0.38	0.51	−32.55
Seeds per plant						
C	1302.73	−763.10	256.84	−2.07	0.68	163.57
S	−105.63	275.67	98.78	0.14	0.63	−39.30
Empty pods						
C	0.41	−0.38	3.15	−1.83	0.01	12.93
S	2.37	−4.53	5.71	1.65	−0.61	66.76
Seed yield						
C	35.39	−22.35	10.67	5.95	0.55	149.29
S	1.45	6.79	3.11	0.34	0.73	12.81
100 Seed weight						
C	44.79	−16.38	31.47	−1.11	0.52	74.80
S	17.59	44.55	23.74	4.15	0.72	20.49
Na in stems						
C	357.05	−95.58	96.29	−3.99	0.73	99.24
S	−5535.70	22763.98	2584.32	1.05	0.87	−27.93
Cl in stems						
C	3178.40	3129.80	5029.21	0.92	0.56	28.03
S	78017.39	56307.06	48004.72	0.27	0.74	42.79
K in stems						
C	26189.77	−2545.39	11636.67	−2.03	0.67	74.56
S	12030.20	2363.53	9400.15	4.39	0.61	50.56

heritabilities (calculated from additive and environmental variances) under control conditions. However, the values of narrow-sense heritabilities in control conditions appear to be overestimated due to counteracting effects of additive and dominance genes with high magnitudes of dominance and additive variances. The negative signs for narrow-sense heritabilities observed for these traits under saline treatment indicate the reducing effect of additive genes under saline treatment.

For Na and Cl concentrations in stems, broad-sense heritability values were higher in the salinity

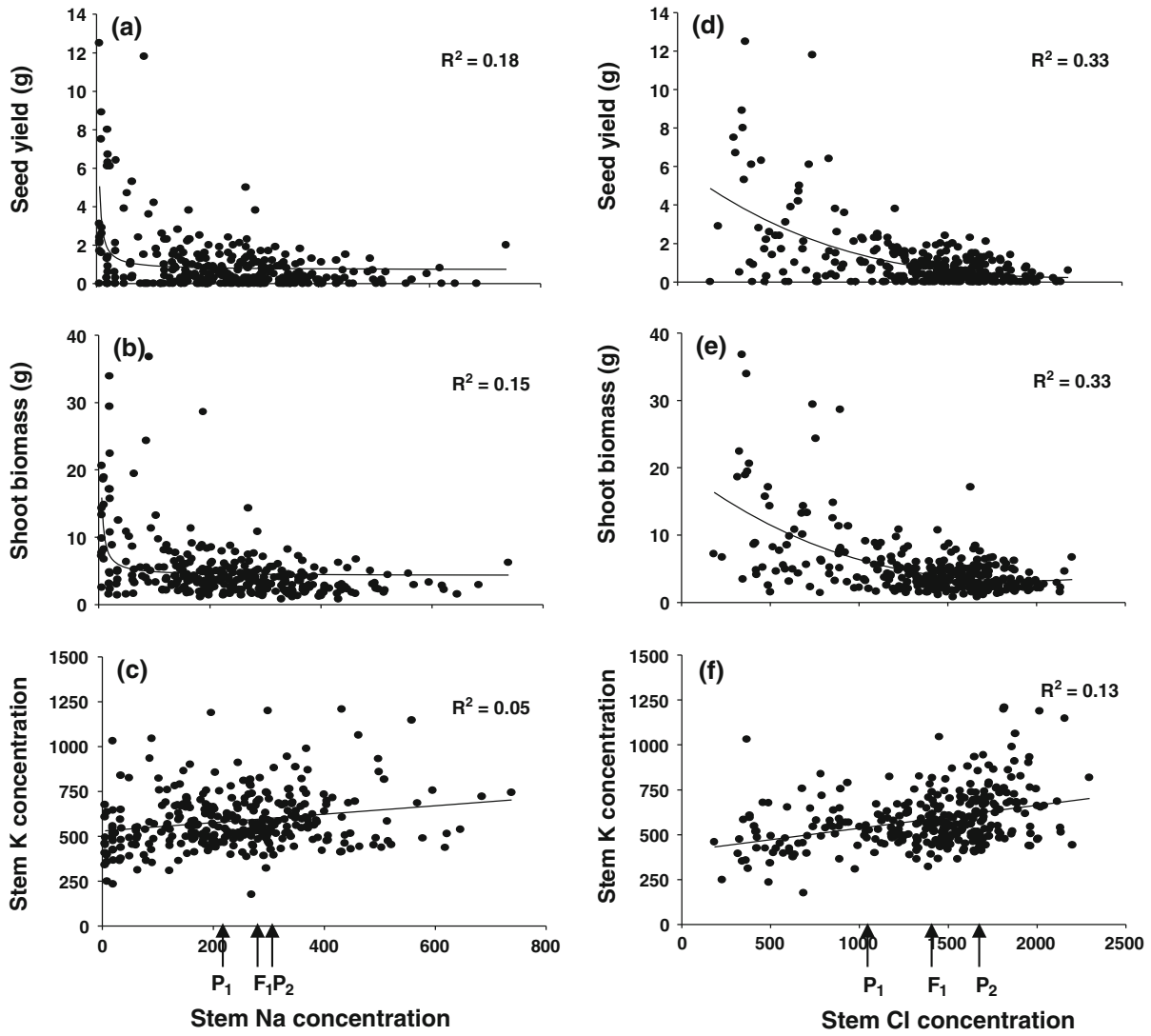

Fig. 1 Relationships between stem Na concentration from F_2 segregants grown in salt treatment and **a** seed yield (polynomial inverse 1st order), **b** shoot biomass (polynomial inverse 1st order), and **c** stem K concentration (linear). Relationships between stem Cl concentration and **d** seed yield (cubic), **e** shoot biomass (cubic), and **f** stem K concentration (linear). Ion concentrations expressed in μmol g^{-1} weight. *Arrows* indicate the average values of respective populations. Information about the controls is given in the "Results" section

treatment than the control and the opposite was true for K (Table 3). The Narrow-sense heritability values under salinity were lower for Na and K, and higher for Cl, compared to the control. The additive component contributed a large proportion of the variation in Cl and K concentrations under salinity and dominance variance was higher for Na. Over dominance was observed for Na and K in both control and salinity treatments, whereas Cl had almost complete dominance in the control and partial dominance in the salinity treatment (Table 3).

The salt-sensitive parent (ICC 6263) has white flowers and the tolerant parent (ICC 1431) has pink flowers. ICC 6263 was used as the female parent in the cross, and F_1s had pink flowers. In F_2, pink and white flowers segregated in the 3:1 ratio with the χ^2 value of 0.32 ($P > 0.01$). When F_1 plants were backcrossed with the sensitive parent (ICC 6263, white flowers) the resulting progenies were pink or

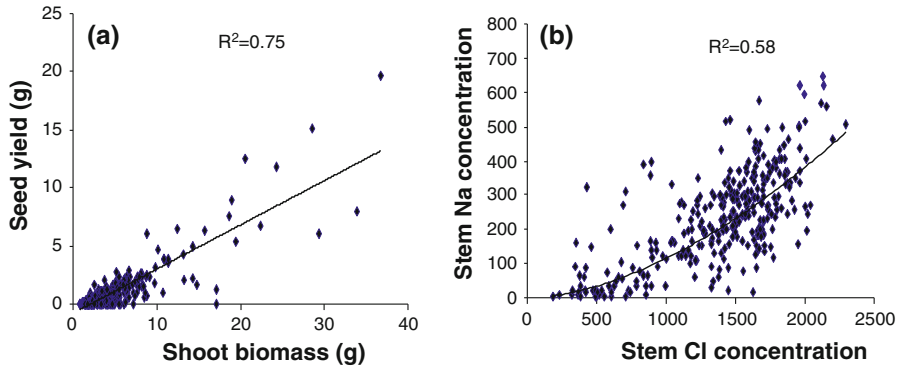

Fig. 2 Relationships of F_2 segregants grown in salt treatment between **a** seed yield and shoot biomass (linear), and **b** stem Na and stem Cl concentrations (cubic). Ion concentrations expressed in µmol g^{-1} dry weight. Information about the controls is given in the "Results" section

white flowered that segregated in 1:1 ratio with a χ^2 value of 2.14 ($P > 0.05$). Progenies from a backcross to the tolerant parent (ICC 1431, pink flowers) all produced pink flowers.

Discussion

This experiment confirmed the findings of Vadez et al. (2007) that ICC 6263 is more salt sensitive than ICC 1431; ICC 6363 had less pod and seed numbers, and less seed yield in saline soil. Sensitive lines in the populations tested also produced less pods and seeds, and had lower 100-seed weight, as compared with the tolerant lines in saline conditions. However, although the sensitive parent had more empty pods in saline conditions than the tolerant parent, this was not evident within the populations tested (Table 1). The reductions in salt tolerance in F_1, F_2 and BC_1P_1 populations indicate that genes contributing to higher yields in saline soil from the tolerant parent (ICC 1431) were recessive to those responsible for low yields in saline soil from the sensitive parent (ICC 6263). Even though additive gene effects were more significant than the dominance effects (Table 2), the variances observed acted in opposite directions, resulting in low heritability estimates under saline conditions (Table 3). The degree of dominance ranged from partial dominance (0.28 in days to maturity) to overdominance with the highest degree of dominance being 4.43 for 100-seed weight in the salinity treatment (Table 3). With two exceptions in the control treatment, all values ranged from partial to complete dominance. Gardner (1963) suggested that values in early generations tend to be overestimated due to an upward bias from the repulsion phase of linkage and, in further generations, the linkage will be broken due to recombination and a low degree of dominance. Such dominance of lower yields in F_1 chickpea hybrids was also observed in different diallel crosses between relatively salt tolerant and sensitive lines (Ashraf and Waheed 1998).

Interestingly, no gene interaction was observed in either control or salinity treatments for pods per plant, seeds per plant, or seed yield. In the control treatment, these traits were controlled by dominant effects whereas under salinity only additive effects were significant. This clearly benefits selection of breeding methodology and trait improvement under salinity. Additive gene effects are fixable, and therefore selection for traits controlling such effects is very effective. In contrast, a previous study, conducted in a controlled environment, found that dominance effects controlled chickpea yield traits (e.g. pods per plant, seeds per plant and seed yield) under 40 mM NaCl salinity (Ashraf and Waheed 1998). These different conclusions might be due to differences in salt-types and concentrations used, different genetic backgrounds, and/or screening environment used (controlled environment or outdoors). In chickpea, it has been reported that low relative humidity (55%) coupled with NaCl (36 mM) killed most plants and tolerance varied between genotypes with changes in humidity (75 and 95%) levels (Lauter and Munns 1987). Thus, the high influence of environmental factors on different traits under salinity likely influenced outcomes of studies on salinity tolerance in chickpea (Flowers et al. 2010).

Epistatic interactions were significant for most of the traits measured, the exceptions were pods per plant, seeds per plant, and seed yield traits (Table 2) and these have been discussed in the preceding

paragraph. For all other traits, except for shoot biomass in the control, estimates of dominance by dominance effects [l] were significant and opposite in sign to those of dominance effects alone [h], indicating the presence of a duplicate type of epistasis (15:1) for these various traits. Existence of such epistasis and higher magnitudes of [h] and [l] in the population generally reduces efficiency of selection. Usually selection would be effective after several generations once a high level of gene fixation is attained for the traits showing significant gene interactions.

Signs associated with different estimates of epistasis indicate the direction in which gene effects influence the population mean. Mather and Jinks (1982) proposed the association or dispersion of genes in the parents based on signs associated with epistatic gene effects such as additive by additive [i] and additive by dominance [j]. These signs were in opposite directions and significant in the control for days to flowering and shoot biomass, and in saline conditions for 100-seed weight. A negative sign for any of these parameters indicates an interaction between increasing and decreasing alleles, thus providing some evidence for the existence of dispersion in the parental genotypes which hinders early selection for such traits. Similarly, signs of these parameters were both negative for stem Na and K concentrations in the control which suggests a large influence of the recessive parent. Under salinity, a positive sign for these two parameters suggests that further improvement is possible with selection, but no other traits in the saline treatment had significant positive sign for [i] and [j]. Such dispersion with more recessive genes compared to dominant genes has been observed in chickpea evaluated at 40 mM NaCl salinity (Ashraf and Waheed 1998).

Relationships between stem ion concentrations and shoot biomass and seed yield were explored in F_2 populations under salinity. Shoot biomass and seed yield showed a non-significant polynomial inverse 1st order fits with Na concentration, and a significant cubic relationship was observed with Cl (Fig. 1d, e). Thus, differences in shoot biomass and seed yield might be explained partially by Cl concentration in stems (both with $R^2 = 0.33$), although Na and Cl concentrations in stems also showed a significant positive relationship ($R^2 = 0.58$). By contrast, a recent large-scale screening of salinity tolerance in chickpea did not find relationships between shoot Na or K at 50 days after sowing with seed yield at maturity (Vadez et al. 2007), but tissue Cl was not assessed. However, other studies in chickpea indicated that shoot dry weight had a strong negative relationship ($R^2 = 0.90$ in Cl salinity; $R^2 = 0.75$ in SO_4^{2-} salinity) with shoot Na, whereas the relationship with shoot Cl ($R^2 = 0.33$) was weaker (Lauter and Munns 1986, 1987); these studies did not evaluate relationships with seed yield. Interestingly, in the present study the tolerant (ICC 1431) had 57% higher Cl in stems than the sensitive (ICC 6263) parent (Table 1); sequestration of Cl in the stems may reduce Cl entry into leaves (cf. Cl unloading into sheaths of *Sorghum bicolor*; Boursier and Lauchli 1989), but we can only speculate upon this possibility as leaf Cl was not determined in the present study of chickpea. In F_2 populations, more than 70% of segregants had Cl concentrations between 1,300 and 1,700 μmol g^{-1} dry weight (nearer to the tolerant parent) and the Cl concentration in F_1, F_2 and BC_1P_2 (backcross population with the tolerant parent) had intermediate levels between the two parents (Table 1). This intermediate expression was mainly caused by the presence of higher additive gene effects in controlling the trait (Table 2).

As phenology can influence plant responses to abiotic stresses such as salinity, the present study used parents of similar phenology. Salinity delayed flowering by 2–8 days amongst the populations tested here; earlier studies of chickpea also found that salinity delayed flowering (Bishnol et al. 1990; Vadez et al. 2007). The length of the flowering period can also be reduced by salinity (Dhingra et al. 1996), and in the present study since days to maturity under salinity and control were similar so the reproductive period (flowering or pod formation) was shortened by salinity. This shorter reproductive period would likely have contributed to yield reduction in saline conditions.

In conclusion, the present study confirms the adverse effects of moderate salinity on chickpea and that genotypes differ in salt tolerance. Yield traits such as pods per plant, seeds per plant and seed yield were controlled by additive effects, which suggest scope for breeding and selection for improved salinity tolerance in chickpea. In the present cross, influence of the sensitive parent was reflected more in non-yield-related traits due to its dominant nature in early generations; hence selection for such traits under

salinity would be more effective in later filial generations as useful genes will be fixed due to breakage of unfavorable linkages. The parental lines used in this study were chosen based on their differences in salinity tolerance (Vadez et al. 2007) but these are not as high yielding as recent cultivars. Development of high-yielding salt tolerant cultivars of chickpea will require introgression of salinity tolerance into suitable, modern agronomic backgrounds.

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