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Salt sensitivity in chickpea

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ABSTRACT

The growth of chickpea (*Cicer arietinum* L.) is very sensitive to salinity, with the most susceptible genotypes dying in just 25 mM NaCl and resistant genotypes unlikely to survive 100 mM NaCl in hydroponics; germination is more tolerant with some genotypes tolerating 320 mM NaCl. When growing in a saline medium, Cl^-, which is secreted from glandular hairs on leaves, stems and pods, is present in higher concentrations in shoots than Na^+. Salinity reduces the amount of water extractable from soil by a chickpea crop and induces osmotic adjustment, which is greater in nodules than in leaves or roots. Chickpea rhizobia show a higher 'free-living' salt resistance than chickpea plants, and salinity can cause large reductions in nodulation, nodule size and N_2-fixation capacity. Recent screenings of diverse germplasm suggest significant variation of seed yield under saline conditions. Both dominance and additive gene effects have been identified in the effects of salinity on chickpea and there appears to be sufficient genetic variation to enable improvement in yield under saline conditions via breeding. Selections are required across the entire life cycle with a range of rhizobial strains under salt-affected, preferably field, conditions.

Key-words: chloride; *Cicer arietinum*; flowering; germination; N_2-fixation; plant breeding; plant water relations; sodium; soil salinity.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) was one of the earliest grain crops cultivated by humans. Today, chickpea ranks third (FAO 2008) among food legumes for world production, behind beans (*Phaseolus* spp.) and field pea (*Pisum sativum* L.). Although more than 50 countries are reported to grow chickpea, only 22 cultivate more than 20 000 ha; 19 cultivate 10 000 to 20 000 ha. Total annual world production is 8.4 million tonnes, and the major chickpea producing countries include India (65% of annual production), Pakistan (10%), Turkey (7%), Iran (3%), Myanmar (2%), Mexico (1.5%) and Australia (1.5%) (FAO 2008). Other producers include Ethiopia, Iraq, Israel, Jordan, Morocco and Syria; Canada, Tanzania and Malawi are emerging as chickpea producers.

Two major types of chickpea are recognized, *desi* and *kabuli*. The *desi* type is generally small-seeded (less than 200 mg per seed) with coloured seed coats and an angular seed shape. The *kabuli* type is generally large-seeded (more than 350 mg per seed) with beige or cream-coloured seed coats and a 'rams-head' shape. The two types can be hybridized, but there are strong consumer and culinary preferences for *desi* and for *kabuli* chickpea. At 21% protein (range 17–26%), chickpea seed is a protein-rich supplement to cereal-based diets, especially critical in developing countries where people either cannot afford animal protein or are vegetarian by choice. In addition to its importance in human food and animal feed, chickpea plays an important role in sustaining soil fertility by fixing up to 140 kg N ha^{-1} $year^{-1}$ (Rupela 1987). Thus, chickpea is a low-input-requiring crop, deriving over 70% of its N requirement through symbiotic N_2 fixation and providing benefits for following cereal crops (Siddique *et al.* 2005). Chickpea is mainly grown as a cool-season crop under both rainfed (>90%) and irrigated conditions, often maturing in the driest and hottest part of the year. Major biotic constraints to chickpea production include diseases such as fusarium wilt (*Fusarium oxysporum f.* sp. ciceri), ascochyta blight (*Ascochyta rabiei*), botrytis grey mould (*Botrytis cinerea*); and pests such as *Helicoverpa* pod borer (*Helicoverpa armigera* and *H. punctigera*) and leaf miner (*Liriomyza cicerina*) (Nene & Reddy 1987; Reed *et al.* 1987). Among abiotic constraints, drought, chilling temperatures and soil salinity limit the productivity of chickpea.

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Table 1. Germination percentages in sensitive and resistant (at germination) genotypes of chickpea. More examples are provided in Table S1

Germination (%)			
Sensitive genotypes	Resistant genotypes	Conditions	Source
15% Pusa-256	100% Pusa-312	85 mM NaCl in agar	Saxena & Rewari (1992)
12% CM-72	55% C-727	NaCl solutions at 6 dS m^{-1}	Khalid *et al*. (2001)
0 ILC-3279	69% ILC-205	NaCl+Na_2SO_4 solutions at 32 dS m^{-1}	Zurayk *et al*. (1998)

Soil salinity affects about 80 million ha of arable lands worldwide (see Munns & Tester 2008), and this area is expanding. Estimates of yield losses of chickpea due to soil salinity are not available; however, considering its sensitivity (described in a later discussion) and that the salt concentration in the soil increases as the soil dries at the end of the growing season (Rengasamy 2006), we suggest annual salt-induced yield losses of 8 to 10% globally are likely in chickpea. In this review we analyse what is known of the effects of salinity, but not those of sodicity, on chickpea. We have focused on chickpea because salt sensitivity in this crop has not been previously reviewed in detail and have generally excluded comparisons with other legumes for reasons of space. We describe the responses in terms of resistance rather than tolerance as chickpea is highly sensitive to salinity when compared to other species in cropping systems (e.g. bread wheat, see Dang *et al*. 2008). Salinity affects germination, plant establishment, N_2-fixation, growth, flowering, pod formation and retention and seed filling.

GERMINATION

Salinity (as NaCl) reduces both the rate and extent of germination of chickpea seeds (Table 1), although there is large variation amongst genotypes.[1] For example, ILC-482 took 8 d to reach 70% germination in 120 mM NaCl, whereas Barkla took 10 d to reach just 40% germination in the same salt concentration (in the absence of salt, ILC-482 reached 97% germination in 6 d, whereas Barkla took 8 d to reach 96% germination; Esechie, Al-Saidi & Al-Khanjari 2002). Some genotypes will germinate in NaCl concentrations whose electrical conductivity[2] (EC) is 32 dS m^{-1} (i.e. around 320 mM NaCl) whereas germination of other genotypes is reduced to 15% by half that salt concentration (Table 1; see also Table S1). Whether such differences can be related to seed mass or type (*kabuli* versus *desi*) has not been rigorously tested although a comparison between one *kabuli* and one *desi* genotype (Jam and Kaka, respectively) revealed no difference in the effects of salinity on germination (Soltani *et al*. 2002). Within three *kabuli* genotypes (AKN-97, Gokce and Uzunlu-99), the mean time of small seed to germinate (7 mm) at relatively high solution salinity (16.3 dS m^{-1}) was shorter (3.8 d) than of large seed (9 mm; 4.7 d); all three genotypes showed 100% germination at this salinity (Kaya *et al*. 2008, in the absence of salt, the mean time of the small seed to germinate was also slightly shorter at 2.7 d than for large seed, which was 3.3 d).

Tests of germination have commonly been performed using NaCl or NaCl plus Na_2SO_4, and sometimes, in more complex mixtures, with $CaCl_2$ and $MgCl_2$ (see Table S1). There is evidence that Na_2SO_4 is more inhibitory to germination than NaCl, KCl or K_2SO_4, although, as far as we are aware, the effect of all these different salts has only been compared on a single genotype (C-214) (Sheoran & Garg 1983); Table S1). With mixed salts, as with single salts, there is a large genotypic variation in the effect of concentration on germination, whether in solution or soil (Dua 1992; Kathira *et al*. 1997). Some genotypes are able to germinate at 32 dS m^{-1} (e.g. ILC-205 and ILC-206, Zurayk *et al*. 1998), while for others, the rate and percentage germination can be reduced in an EC of just 4 dS m^{-1} (Mer *et al*. 2000, salinity was adjusted by adding a mixture of NaCl, Na_2SO_4 and $CaCl_2$ in the ratio 2:1:1). The speed of germination is, as reported for single salts, slowed by mixed salts (e.g. Yadav *et al*. 1989; Dua 1992), although the final percentage germination is not always reduced (Dua 1992). Emergence from salinized soil (irrigated with a saline solution) has been shown to be slower and with a lower final percentage than germination in solutions at similar salinities (Esechie *et al*. 2002), indicating that early seedling growth might be more salt-sensitive than radical emergence. The effect of seed priming on the ability to germinate in saline conditions has not been investigated for chickpea.

Virtually nothing is known of the reasons for the differences between chickpea genotypes in their abilities to germinate in saline conditions. The presence of concentrations of NaCl (200 mM) that prevent germination in the genotype

[1]Here, we refer to cultivars and genotypes by the names used in the original publications. We provide synonyms for the ICRISAT Chickpea Collection (ICC-) and/or ICARDA Legume Chickpea (ILC-) in Table S5.

[2]The electrical conductivity (EC) of a solution depends on the concentration and nature of the salts in that solution. Where the solution is generated from a water-saturated paste of the soil, we use the abbreviation EC_e. An empirical approach has been used to generate the equation log C = 0.955 + 1.039log EC [e.g. a 48 mM NaCl solution has an EC of 5 dS m^{-1}]. For many purposes, it is sufficient to approximate C ≈ 10 * EC, where C is the concentration in mM and EC is measured in dS m^{-1} [Tanji (1990) *Agricultural Salinity Assessment and Management*. American Society of Civil Engineers, New York].

Castellana also modified the patterns of protein synthesis in these seeds (Colorado *et al*. 1994; Colorado, Nicolas & Rodriguez 1995) and reduced the expression of a gene encoding calmodulin, an action similar to that of abscisic acid (ABA) (50 μM), which also inhibited germination (Nicolas *et al*. 1998). It is not clear, however, whether the effects observed were acclamatory or pathological, as the salt concentration used was high, but not higher than that in which some genotypes can germinate (Zurayk *et al*. 1998); incorporation of leucine into protein declined after 36 h, but whether seed would germinate if transferred back to non-saline conditions was not tested.

Summary of the effects of salinity on germination

In conclusion, it is clear that while there is a large variation in the resistance of germination of chickpea to saline conditions, little is known of the reasons. Generally, it is not clear from the published data whether or not seeds that do not germinate during relatively short-term salinity treatments are still viable. Because low and slow germination could reduce yield, the response of germination to salinity should be taken into account in the choice of variety for planting by farmers and in breeding programmes. Tests of germination would best be made in soil salinized to known salinities in the laboratory rather than in solutions in Petri dishes (see previous discussion, Esechie *et al*. 2002) and should include emergence.

VEGETATIVE GROWTH

Of about 110 research papers that have been reviewed, 30 reported the effects of salinity early in the development of the plant (germination or seedling stage), 40 evaluated the effect of salinity from about 30 d onwards but did not continue until the plants reached maturity and 30 evaluated yield and/or yield parameters under saline conditions. A third (23) of the 70 studies that assessed chickpea response to salinity for at least 30 d after sowing were carried out in sand culture that was artificially salinized with a mixture of salts and a similar number of studies used hydroponic culture solutions (19) or soil-filled pots (20). Only a small number of studies (8) were carried out in the field.

Growth reductions can be severe in chickpea when exposed to NaCl levels that might be regarded as moderate for most crops. Serraj, Krishnamurthy & Upadhyaya (2004) report a 60% biomass reduction at 40 d after sowing, averaged across 234 chickpea accessions grown in a Vertisol treated with 80 mM NaCl solution. The most sensitive genotypes do not survive in NaCl concentrations as low as 25 mM (Table 2), while the most resistant genotypes are unlikely to grow in 100 mM NaCl in hydroponics (and at lower concentrations if the humidity were low). Several studies reported that root development is more sensitive than shoot development (Ashraf & Waheed 1993; Dua 1997; Tejera, Soussi & Lluch 2006).

From the available data, it is difficult to conclude whether the different systems used to grow chickpea affect relative performance of different genotypes, since few genotypes are common to different experiments. L-550 (reported as resistant) and E-100 (sensitive) were similarly ranked in hydroponics (Lauter & Munns 1987), vermiculite (Soussi, Lluch & Ocana 1999; Tejera *et al*. 2006) and salinized soil (Vadez *et al*. 2007). Vadez *et al*. (2007) also found good agreement for the resistance of CSG-8962 with a previous study (Dua & Sharma 1995) carried out in different soil. The salt sensitivity of chickpea genotypes does, however, depend on soil type with greater reductions in growth and yield in a sandy soil than in a clay soil with the same concentration of added NaCl. Nevertheless, preliminary results suggest that the ranking in salt sensitivity of genotypes is largely independent of soil type (L. Krishnamurthy, V. Vadez, N.C. Turner *et al.* unpublished results 2009). So this limited assessment would suggest that the growth system

Table 2. Effect of salinity on the vegetative growth (dry matter expressed relative to growth under control conditions) of genotypes of chickpea grown in a variety of conditions. Salt treatments were applied from the time of sowing and biomass data collected at different vegetative stages

Relative growth, genotype and reputation for response to salt (% control)			
Sensitive	Resistant	Salt treatments	Source
39% ICC-6263	35% ICC-1431	1.17 g NaCl kg^{-1} soil in a Vertisol	Vadez *et al*. (2007)
36% ICC-12908	93% CM-663 68% ICC-10572	40 mM NaCl, hydroponic sand culture	Ashraf & Waheed (1993)
73% H-208	90% H-355	6 dS m^{-1} sulphate-salinized soil[a]	Manchanda & Sharma (1989)
83% shoot, 30% root CSG-8890	61% shoot 91% root CSG-88101	7.8 dS m^{-1} with mixed salts in hydroponics	Dua (1998)
75% Pusa-209	95% BG-312	40 mM mixed salts in hydroponic sand culture	Sharma & Kumar (1990)
50% Sirio	85% L-550	100 mM NaCl in hydroponic culture in vermiculite	Tejera *et al*. (2006)
0% E-100	70% L-550	25 mM NaCl in hydroponics at 55% relative humidity	Lauter & Munns (1987)

[a]Straw yield at maturity.

does not affect relative performance of different genotypes, although further work is required to confirm this view.

Physiological changes in chickpea grown in the presence of salt

Photosynthetic pigments decreased in concentration in chickpea grown in NaCl (100 mM) (Datta & Sharma 1990; Beltagi 2008), and photosynthesis was reduced by 60% (Murumkar & Chavan 1993); genotypes have also been shown to differ in the effects of salinity on chlorophyll fluorescence (Epitalawage, Eggenberg & Strasser 2003). Salinity can increase senescence in chickpea (Katerji *et al*. 2001) and induce the production of ethylene and its precursor 1-aminocycloprane-1-carboxylic acid (ACC) in roots and nodules (Kukreja *et al*. 2005; Nandwal *et al*. 2007). In both roots and nodules, ACC increased three-fold and ethylene doubled with exposure for 3 d to 10 dS m^{-1} of mixed salts (where the molar ratios of Na^+, Ca^{2+} and Mg^{2+} were: $Na^+ : Ca^{2+}$ 1:1; $Ca^{2+} : Mg^{2+}$ 1:3 and $Cl^- : SO_4^{2-}$ 7:3) (Kukreja *et al*. 2005; Nandwal *et al*. 2007). Symptoms of leaf necrosis, presumably related to the destruction of chlorophyll in leaf cells resulting from ion toxicity when Na^+ and/or Cl^- exceed tolerable levels in tissues, were reported by Maliro *et al*. (2008) and these authors showed that 'visual scores' of necrosis could be used as an index of resistance.

The presence of salinity (up to 10 dS m^{-1} of mixed salt, see earlier discussion) induced an increase in hydrogen peroxide of 180% in chickpea (CSG-8962) roots and of lipid peroxidation by 170% (Kukreja *et al*. 2005). However, in a separate study on chickpea (Gökçe) subjected to 100 mM NaCl for 4 d in hydroponics, although hydrogen peroxide increased (by 170%) in leaves, it decreased in roots (by 20%, Eyidogan & Oz 2007). Reactive oxygen species need to be scavenged for normal growth (Sairam, Tyagi & Chinnusamy 2006) and antioxidant enzymes in chickpea increased in activity and expression under salt stress (Hernandez-Nistal, Dopico & Labrador 2002; Kukreja *et al*. 2005; Eyidogan & Oz 2007). The antioxidant enzyme, superoxide dismutase, increased by 150%, while other enzymes also increased in roots of 60-day-old chickpea exposed to 10 dS m^{-1} (mixed salts, see previous discussion) for 3 d: peroxidase by 220%, ascorbate peroxidase by 240%, glutathione transferase by 140%, glutathione reductase by 126% and catalase by 360% (Kukreja *et al*. 2005). However, the changes in enzyme activities did not prevent membrane damage in roots (Kukreja *et al*. 2005) or leaves (Eyidogan & Oz 2007), which increased significantly as measured by malondialdehyde content as a result of 2 d to 4 d exposure to salinity. We do not, however, know of any study that has evaluated whether genotypic differences in antioxidant production can be related to growth and yield in chickpea.

Mineral concentrations in tissues

The concentrations of Na^+ and Cl^- have been shown to increase in chickpea on exposure to salt (Table 3), with

Table 3. Some examples of Na^+ and Cl^- concentrations in shoots, leaves and roots of chickpea grown under saline conditions

Method salts	Source of nitrogen	Genotype and plant parts	External salt (mM) or dS m^{-1}	Na^+ (μmol g^{-1} dry mass)	Cl^- (μmol g^{-1} dry mass)	[Cl/Na]	Treatment period (d) (Harvest, d)	Source
Hydroponics	NH_4NO_3	UC-5 Shoot	31 mM	380	1510	4.0	53 (53)	Lauter *et al*. (1981)
NaCl	N_2-fixation		31 mM			1.7		
Hydroponics	Nitrate	L-550 Shoot	Pooled over	176	434	2.5	41 (44)	Lauter & Munns (1986a)
$NaCl + Na_2SO_4$		UC-5 Shoot	10, 20, 30 & 50 mM			2.0		
Sand	N_2-fixation	Pusa-209 Leaves	8 dS m^{-1}	830	1970	2.1	20 (50)	Sharma & Kumar (1992)
$Na^+ : Ca^{2+} : Mg^{2+}$ (5:2:3)		Pusa-209 Root		2500	1840	0.7		
$Cl^- : SO_4^{2-}$ (7:1)								
Soil	NH_4NO_3	4 genotypes[a] Shoot	4 dS m^{-1}	691[a]	2581[a]	3.8[a]	28 (28[b])	Mamo *et al*. (1996)
NaCl		Roots		590[a]	1085[a]	1.9[a]		
Sand	Hoagland[b]	CSG-8890 Shoot	4 dS m^{-1}	3141	8062	2.6	30 (60)	Dua & Sharma (1997)
$Na^+ : Ca^{2+} : Mg^{2+}$ (5:2:1)	(NO_3^- ca. 1.5 mM;	CSG-88101 Shoot		1476	7994	5.4		
	NH_4^+ ca. 0.25 mM)							
$Cl^- : SO_4^{2-}$ (4:1)		CSG-8927 Shoot		1563	6671	4.3		
Hydroponics	Hoagland[b]	CSG-8890 Shoot	7.8 dS m^{-1}	460	1398	3.0	18 (38)	Dua (1998)
NaCl : $CaCl_2$	(NO_3^- ca. 3 mM;	CSG-8890 Root		1062	1226	1.2		
$2H_2O : MgCl_2$	NH_4^+ ca. 0.5 mM)	CSG-88101 Shoot		434	1757	4.0		
$6H_2O$ (2:1:2)		CSG-8890 Root		589	1896	3.2		

[a]DZ-local, Mariye, DZ-10-9-2 and DZ-10-16-2, pooled across genotypes.
[b]Treatment applied in two parts over 3 d.

tissue Cl^- concentrations generally exceeding those of Na^+. Increases in plant ions concentration are not novel and occur with all plants exposed to NaCl. What is generally not clear, however, is at what concentration and in what plant parts ions reach toxic concentrations and whether toxicity can be assigned to Na^+, Cl^- or SO_4^{2-} (SO_4^{2-} can be high in some saline soils). These are important questions as knowledge of plant ion concentrations can be used to inform breeding strategies. For example, is poor performance under saline conditions correlated with ion concentrations in shoots or in particular leaves? Do genotypes vary in the transport properties of these ions in their root systems? Do environmental factors other than salinity modulate the transport of ions from roots to shoots? Does the source of nitrogen (N) alter transport of Cl^- and/or Na^+? For chickpea, there are at least partial answers to some, but not all, of these questions.

There is evidence that after exposure of chickpea to relatively low concentrations of NaCl (less than 50 mM), growth is unaffected for a period of days while shoot ion concentrations rise to a value at which growth is reduced to a new quasi-steady state. Lauter & Munns (1987) reported growth (expressed relative to that of non-saline controls) of two varieties (L-550 and E-100) of chickpea to be unaffected over 10 d in 30 mM Na^+ (15 mM NaCl and 7.5 mM Na_2SO_4) until shoot Na^+ concentrations reached 560 μmol g^{-1} dry mass and shoot Cl^- was 676 μmol g^{-1} dry mass. When exposed to different concentrations of NaCl or Na_2SO_4 (10, 20, 30 or 50 mM) in hydroponics, growth was negatively correlated with Na^+ concentration in the shoot from 50 to 550 μmol Na^+ g^{-1} dry mass with little difference between SO_4^{2-} and Cl^- treatments at equal Na^+ concentrations (Lauter & Munns 1986a). Similarly, Dua & Sharma (1997) also found a negative correlation between shoot growth, expressed relative to the control, and shoot Na^+ concentrations between 400 and 4000 μmol g^{-1} dry mass of three genotypes of chickpea in sand culture. Such relationships may depend on a critical level of Na^+ being accumulated within the shoots. Data reported by Richter *et al*. (1999) do not demonstrate any relationship between vegetative growth or grain yield and shoot Na^+ or Cl^- concentration, but maximum Na^+ concentrations were under 200 μmol g^{-1} dry mass of shoots. The variability in ion concentrations shown in Lauter & Munns (1986a) suggests that no significant effect of shoot Na^+ or Cl^- concentration on relative shoot dry mass would be discernible until shoot ion concentrations were higher than 100 μmol g^{-1} dry mass. A further problem in correlating yield with ion concentrations for highly sensitive species is that yields can be so low that correlations are not apparent (e.g. in Ashraf & Waheed 1993). Whatever the reasons, an assessment of 263 germplasm entries for shoot dry mass and Na^+ concentration in the shoot at 40 d after sowing, showed no relationship between these two parameters ($R^2 = 0.04$, L. Krishnamurthy, V. Vadez, N.C. Turner *et al.* unpublished results 2009). In this study, most genotypes had Na^+ concentrations in shoots below 174 μmol g^{-1} dry mass of shoots and few (8 of 263) had Na^+ concentrations between 174 and 260 μmol g^{-1} dry mass of shoots.

While the data presented by Lauter & Munns (1987) indicated growth to be more closely related to shoot Na^+ than shoot Cl^- concentrations (see also below), other evidence suggests Cl^- to be the more important ion as far as toxicity is concerned. Across four genotypes grown for 7 weeks at 4 dS m^{-1} created in soil by adding NaCl alone, reduction in growth appeared related to shoot Cl^- rather than shoot Na^+ concentration (Mamo, Richter & Heiligtag 1996). Cl^- concentrations in leaves are commonly higher than those of Na^+, when expressed on a molar basis (Table 3), but the difference is smaller for roots than leaves (Mamo *et al*. 1996; Dua 1998) or even reversed (Sharma & Kumar 1992). The presence of SO_4^{2-} and perhaps the fact that the external Na^+ was twice the concentration of Cl^- in the treatments used by Lauter & Munns (1987) may be the reason for the poor correlation between growth and shoot Cl^- in their data.

A notable feature of the leaves of chickpea is the presence of secretions on the surface. These secretions arise from glandular hairs or trichomes that are present on leaves, stems and pods (see Lazzaro & Thomson 1989). Unusually amongst trichomes, secretions are dominated by organic acids (Lazzaro & Thomson 1989), and the pH can be as low as 1 (Lauter & Munns 1986b). When plants were grown in a mixture of NaCl and Na_2SO_4 (half Na^+ as Cl^- and half as SO_4^{2-}), the concentration of Cl^- in the secretion was approximately 231 mM while Na^+ did not exceed about 60 mM (Lauter & Munns 1986b). Thus, at least part of the difference between the concentrations of Na^+ and Cl^- recorded for shoots (see Table 3) could be due to differences in the intra- and extra-cellular concentrations of the two ions.

Although tissue ion concentrations are commonly reported on the basis of dry mass, any metabolic effects of salinity are likely to be a consequence of the activity of ions in the cytoplasm and/or vacuole and so be influenced by cellular water content and compartmentation. For two genotypes (CSG-88101 and CSG-8890) with differing salinity resistance, it was notable that resistance was associated with an ability to maintain tissue water content under saline conditions, so mitigating changes in ion concentration (Dua 1998). For example, the shoot Na^+ concentrations after 18 d exposure to salts expressed on a dry mass basis were similar at 460 μmol g^{-1} dry mass in the sensitive genotype (CSG-8890) and 434 μmol g^{-1} dry mass in the resistant genotype (CSG-88101). However, when expressed on the basis of the water content, the concentration of Na^+ in the sensitive genotype (560 mM) was about twice that (223 mM) of the resistant genotype. Cl^- concentrations were about double those of Na^+, being 1398 and 1757 μmol g^{-1} dry mass in sensitive and resistant genotypes, respectively: 1690 mM and 890 mM, respectively, when expressed on the basis of shoot water content. Differences in water content could also confound attempts to find correlations between growth and ion concentrations expressed per unit dry mass.

The effect of salinity is, as might be expected, influenced by environmental factors such as humidity and nitrogen source. In the experiment conducted by Lauter & Munns

(1987) at four relative humidity (rh) values (55%, 75%, 88% and 95% rh) and a range of salt concentrations (0, 12 and 24 mM Na^+ added as equal molar ratios of Cl^- and SO_4^{2-}), genotypic differences in the response to the interaction between salt and humidity were evident. Shoot growth (represented by the log of the shoot dry mass) was linearly related to shoot Na^+ concentration ($R^2 = 0.77$), but poorly ($R^2 = 0.33$) related to shoot Cl^- concentration (Lauter & Munns 1987). Decreasing the atmospheric humidity increased the Na^+ concentration in the leaves of both of the genotypes studied, although the rate of change of leaf Na^+ concentration with decrease in humidity differed between genotypes even though they showed similar overall salt sensitivity. These data indicate that suitable levels of salinity to be used to screen chickpea will depend on the humidity at the screening site and may need re-adjustment from environment to environment or year to year.

The source of nitrogen available to chickpea can also influence shoot ion concentrations. Over 53 d, Na^+ accumulated to 790 μmol g^{-1} dry mass in shoots of nodulated plants (cv. UC-5) growing in an external concentration of just 31 mM NaCl without the provision of inorganic N (Lauter, Munns & Clarkin 1981). Providing N as NH_4NO_3 reduced the accumulation of Na^+ by half, to 390 μmol g^{-1} dry mass. The lower shoot Na^+ concentration in NH_4NO_3-fed plants compared with those reliant on fixed-N might, at least partially, have resulted from 'dilution by growth' in the larger, mineral-N-supplied plants. For Cl^-, however, the situation was quite different: shoots of plants relying on nodules for their N supply contained 1340 μmol Cl g^{-1} dry mass, while those provided with NH_4NO_3 contained even more Cl^- (1510 μmol g^{-1} dry mass). Consequently, Cl^-/Na^+ ratios in the shoots of plants provided with inorganic N were twice those of plants relying on N_2-fixation (Table 3). Other studies, however, failed to find differences in shoot Na^+ concentration between plants reliant on mineral-N or fixed-N (Rao & Sharma 1995b; Baalbaki *et al*. 2000). One study evaluated Pusa-256 inoculated with seven different rhizobial strains (Rao & Sharma 1995b), while the other (Baalbaki *et al*. 2000) involved two chickpea cultivars and two rhizobial strains. In addition to the absence of any effect of nodulation status on shoot Na^+ there was no effect on shoot Cl^- concentration (Baalbaki *et al*. 2000). Furthermore, a non-nodulating cultivar did not differ in shoot Na^+ concentration from four other cultivars with varying degrees of nodulation, when compared within three different salinity treatments (3.2 to 8.1 dS m^{-1}, Rao *et al*. (2002). Thus, there is some discrepancy in the literature over the effects of the N source on shoot Na^+ and Cl^- concentrations in chickpea and further work is required to clarify the situation.

Water relations

Generally, the first response of plants to salinity in the soil arises from an osmotic effect of the NaCl in the root zone with the influence of ions taken up by the plants affecting growth at a later stage (Wilson, Haydock & Robins 1970; Sairam *et al*. 2006; Munns & Tester 2008). Plants commonly respond to the presence of salinity by lowering their osmotic potential through the accumulation of ions and organic compounds in a process known as osmotic adjustment (Bernstein 1961; Flowers & Yeo 1986). With stomata in the leaves opening for entry of CO_2 for photosynthesis, water in the leaves evaporates and is drawn along the soil-plant-atmosphere continuum from the roots and soil. The presence of salt in the root zone lowers (becomes more negative) the water potential in the leaf required to withdraw water from the soil and reduces the water content of the plant in a similar manner to soil drying. With soil drying, leaf growth is reduced and, once a threshold leaf water potential of –0.8 MPa is reached, the stomata begin to close and photosynthesis is reduced in chickpea genotypes that osmotically adjust (see below) (Leport *et al*. 1998; Munns & Tester 2008). Studies in other legumes suggest that stomatal closure results initially from the local synthesis of ABA (Fricke *et al*. 2004), and then the production by the roots of ABA that is transferred to the leaves (Wolfe, Jeschke & Hartung 1990). However, as far as we know, changes in ABA in response to salinity have not been measured in chickpea.

For chickpea, studies in the field have shown that the presence of salt in the soil profile reduces the plant available water capacity; that is the amount of water extractable from the soil by the crop before it reaches maturity (Whish *et al*. 2007; Dang *et al*. 2008). Dang *et al*. (2008) showed that in soils with an EC_e (calculated from a 1:5 extract) of about 10 dS m^{-1} at 0.9 to 1.0 m depth below the surface, chickpea was the most sensitive crop to the subsoil salt among bread wheat, durum wheat, barley, canola and chickpea, because of a greater reduction in water extraction. Based on ridge-regression analysis of a number of ions, they concluded that it was the Cl^- concentration in the soil that was critical (although including exchangeable sodium percentage did improve the regression) and estimated that the critical subsoil Cl^- concentration that reduced chickpea yields by 10% was 490 mg Cl^- kg^{-1} soil. The smaller plant's available water capacity under saline conditions is presumably partly because of restricted root growth of chickpea in saline soil, but also because salt reduces the extraction of water from the soil by the roots. Pot studies where rooting volume was similar in a saline and non-saline soil clearly showed that chickpea was unable to remove as much water from saline soil as from non-saline soil (Sheldon *et al*. 2004). This was presumably due to the lowering of the soil water potential (osmotic effect), the greater energy requirement to extract water from the soil (Rengasamy 2002, 2006; Rengasamy, Chittleborough & Helyar 2003) and the cessation of transpiration at a higher soil water content in saline than in non-saline soil.

Adding 12 or 24 mM NaCl (and lowering the osmotic potential of the solution by –0.06 and –0.12 MPa, respectively) to a hydroponic medium surrounding the roots of chickpea reduced the shoot water potential by 0.22 MPa (with no significant difference between 12 and 24 mM salt or genotypes E-100 and L-550) when measured before shoot growth was affected (Lauter & Munns 1987 – see Table 4).

Table 4. Influence of salinity on the water relations of some genotypes of chickpea

Genotype	Salinity levels	Leaf water potential (-MPa)	Leaf osmotic potential (-MPa)	Calculated bulk turgor pressure (MPa)	Source
C-214	0	0.60	0.94	0.34	Sheoran & Garg (1983)
	5	0.81	1.17	0.36	
	10 dS m^{-1-} (NaCl, Na_2SO_4 KCl, K_2SO_4)	1.15	1.52	0.37	
L-550 and E-100[a]	0	0.71	1.28	0.57	Lauter & Munns (1987)
	12	0.93	1.52	0.59	
	24 mM (NaCl, Na_2SO_4)	0.93	1.52	0.59	
CSG-8962	0	0.47	0.65	0.18	Kukreja *et al*. (2005)
	2.5	0.50	0.76	0.26	
	5	0.59	0.94	0.35	
	10 dS m^{-1} (NaCl, $MgCl_2$, Mg SO_4, $CaCl_2$)	0.61	1.23	0.62	
H-96-99	0	0.44	0.65	0.21	Nandwal *et al*. (2007)
	2.5	0.47	0.75	0.28	
	5	0.52	0.88	0.36	
	10 dS m^{-1} (NaCl, $MgCl_2$, Mg SO_4, $CaCl_2$)	0.56	1.15	0.59	

[a]Pooled data.

A slightly larger decrease than seen in E-100 and L-550 was observed in the leaf water potential of seedlings of the cultivar C-214 exposed to 5 and 10 dS m^{-1} (osmotic potentials of –0.24 and –0.44 MPa, respectively) NaCl for 4 d (Sheoran & Garg 1983 – see Table 4). When the chickpeas were still growing and transpiring at the time that measurements were taken (Lauter & Munns 1987), the decrease in leaf water potential was much greater than the decrease of the osmotic potential around the roots. Further, the decrease in leaf water potential was greater when the relative humidity of the growth cabinets was lowered and the rate of transpiration increased (Lauter & Munns 1987). In the studies by Sheoran & Garg (1983) and Lauter & Munns (1987), the decrease in shoot water potential was accompanied by a decrease in leaf osmotic potential, so that there was no significant change in the bulk leaf turgor pressure, which remained positive (Table 4), suggesting that the leaves adjusted osmotically in response to the salt in the root zone (see below). The maintenance of turgor with the increasing salinization of the rooting medium to 10 dS m^{-1} (an osmotic potential of –0.44 MPa) in the studies by Sheoran & Garg (1983) and Lauter & Munns (1987) strongly suggests that the leaves were accumulating solutes to maintain the osmotic potential (Table 4). However, as tissue water content has also generally been shown to decrease with increasing salinity, particularly in salt-sensitive cultivars (Dua 1998; Singh *et al*. 2005), it is not possible to determine definitely in these studies whether the decrease in osmotic potential in chickpea arose from a concentrating effect of water loss or an accumulation of solutes in the tissues.

Studies by Kukreja *et al*. (2005) and Nandwal *et al*. (2007) measured the relative water content of tissues as well as the osmotic potential of the tissue, so that the degree of osmotic adjustment can be estimated. The relative water content in two chickpea genotypes (CSG-8962 and H-96-99) decreased from 86–87% in the absence of salt (a value that is due to water extraction from cells as a consequence of transpiration) to 73–74% when exposed to 10 dS m^{-1} for 3 d. Calculating the osmotic potential at full turgor indicates that the two genotypes adjusted osmotically by 0.19 MPa at –0.12 MPa (2.5 dS m^{-1}), 0.44 MPa at –0.24 MPa (5 dS m^{-1}) and 0.87 MPa at –0.44 MPa (10 dS m^{-1}). Thus, the decrease in the osmotic potential in the leaves at full turgor was from 50% greater at 2.5 dS m^{-1} to approximately 100% greater at 5 and 10 dS m^{-1} than the decrease in the osmotic potential of the soil solution. Kukreja *et al*. (2005) also measured the osmotic potential and relative water content of the roots, while Nandwal *et al*. (2007) measured the same parameters of nodules. The osmotic adjustment at 100% relative water content was less in roots than leaves, increasing by 0.18, 0.38 and 0.54 MPa at 2.5 dS m^{-1} (–0.12 MPa) 5 dS m^{-1} (–0.24 MPa) and 10 dS m^{-1} (–0.44 MPa), respectively (Kukreja *et al*. 2005). The degree of osmotic adjustment in nodules was greater than in either leaves or roots, increasing by 0.13, 0.51 and 1.25 MPa at 2.5 dS m^{-1} (–0.12 MPa) 5 dS m^{-1} (–0.24 MPa) and 10 dS m^{-1} (–0.44 MPa) salt (NaCl, $MgCl_2$, $MgSO_4$ and $CaCl_2$ where $Na^+ : Ca^{2+}$ was 1:1 and $Ca^{2+} : Mg^{2+}$ 1:3, the $Cl^- : SO_4^{2-}$ ratio was 7:3, all on a molar basis) in H-96-99.

Katerji *et al*. (2005) measured the water relations and osmotic adjustment of a late-maturing drought-sensitive genotype (ILC-3279) and an early-maturing drought-resistant (drought escape) genotype (Filip-87-59C) grown in soil with mean salinities (EC_e) of 0.8, 2.5 and 3.8 dS m^{-1}. The osmotic potential at 100% relative water content, their measure of osmotic adjustment, at the beginning of flowering and podding, was 0.05 and 0.08 MPa lower when grown in soil with a salinity of –0.18 MPa (3.8 dS m^{-1}) than at –0.01 MPa (0.8 dS m^{-1}) in ILC-3279 and Filip 87-59C, respectively. Thus the osmotic adjustment in response to increasing salinity was less than the decrease in the osmotic potential of the soil solution and was less in the putatively drought-sensitive than in the drought-resistant genotype.

Table 5. Proline concentration of shoots and leaves of selected genotypes of chickpea growing under saline conditions (more examples are given in Table S2)

Genotype	Organ	Salinity levels	Proline concentration (μmol g^{-1} fresh mass)	Source
SG-11 DHG-84-11 BG-256 Phule-G-5	Shoots	0, 4, 8 dS m^{-1}	1.07, 1.30, 1.64 1.03, 1.25, 1.63 1.11, 1.17, 1.31 1.17, 1.26, 1.39 *assuming 90% water content	Singh *et al*. (2001)
H-75-35 L-144	Shoots	0, 4, 6, 8,10 dS m^{-1} 0, 4, 6, 8 dS m^{-1}	0.57, 4.1, 6.4, 8.0, 10.5 0.5, 8.2, 11.2, 11.4	Sharma & Kumar (1990)
ILC-1919	Leaves	0, 50, 75, 100 mM	0.12, 0.43, 0.76, 1.22	Soussi *et al*. (1998)
Gökçe	Leaves	0, 100, 200, 500 mM	6.0, 8.1, 2.6, 0.9	Eyidogan & Oz (2007)

Fourteen days later, when the osmotic adjustment was 0.04 and 0.13 MPa in the putatively drought-sensitive and in the drought-resistant genotype, respectively, the decrease in osmotic potential was less than that in the soil solution (Katerji *et al*. 2005). Although the degree of osmotic adjustment was greater in the putatively drought-resistant genotype than the drought-sensitive genotype, this was confounded by the differences in phenology, with osmotic adjustment being greater later in development than earlier in development and therefore greater in the early-maturing drought-resistant genotype (Filip-87-59C) than the late-maturing drought-sensitive genotype (ILC-3279) (Katerji *et al*. 2005). Thus, in summary, the degree of osmotic adjustment is reportedly less in roots than leaves and less in leaves than nodules, but in all cases greater than the change in the osmotic potential of the external solution in sand culture or solution culture studies, but not in salinized soil. However, although chickpea shows osmotic adjustment, its role in salt-sensitive compared with salt-resistant genotypes is not clear from current studies and requires further study.

Any increase in osmotic adjustment under saline conditions is likely a result of an increase in the ions in the vacuole (see preceding section on mineral composition) and the accumulation of soluble sugars. Although total soluble sugars can initially decrease in the leaves of chickpea after 7 d at 75 mM NaCl, the sugar levels more than doubled in 30-day-old chickpea plants after 14 d exposure to 75 mM NaCl (Soussi, Ocana & Lluch 1998). By contrast, total soluble sugars decreased by 3–43% and 12–68% in six chickpea genotypes exposed for 10 d to 4 and 8 dS m^{-1} of NaCl-dominated salinity, respectively (Sharma & Kumar 1990; Singh, Singh & Sharma 2001). In addition to ions and sugars, the concentration of proline increased in leaves, shoots, roots, pod walls, seeds and nodules of chickpea exposed to increasing levels of salinity (Table 5; Table S2). Generally, the concentration of proline in the leaves and shoots doubled on a dry mass basis and increased 10-fold on a fresh mass basis, while the proline level in roots did not increase significantly (Table S2). The only exception to the increase was in salt-sensitive callus, but the concentration of proline did increase in this callus when exposed to NaCl with 10 mM proline added to the medium (Pandey & Ganapathy 1985). The role of proline is controversial. It is a compatible solute and is considered to accumulate in the cytoplasm to balance the accumulation of solutes and ions in the vacuole during osmotic adjustment. Pandey & Ganapathy (1985) argue that proline does not accumulate as a result of stress-induced damage to the cells as suggested by some authors (e.g. Soussi *et al*. 1998), but acts as a protective agent against cellular damage. Screening of chickpea cultivars on the basis of proline accumulation in young plants has given inconsistent results (Chandra 1980, cited in Saxena *et al.* 1994), suggesting that it is not related to salinity resistance in chickpea.

In summary, salinity reduces the plant available water capacity. Under saline conditions, the shoot water potential declines with an accompanying decrease in the solute potential indicating the leaves adjust osmotically in response to salt in the root zone. It appears that at low salt concentrations, changes in the osmotic potential in the leaves maintains leaf turgor and increases the gradient in water potential, whereas at higher salinities the leaf water potential does not decrease by as much as in the external solution. Both ions and soluble sugars accumulate in chickpea shoots under saline conditions, as does proline, although proline accumulation does not appear related to salinity resistance. The extent to which osmotic adjustment reflects changes in leaf water content or solute accumulation *per se* requires further study, as does whether the degree of osmotic adjustment differs between salt-sensitive and salt-resistant genotypes. Since there is some evidence that salt-resistant genotypes are better able to maintain their water content after exposure to salt than salt-sensitive genotypes, it is important that ion concentrations are not only calculated per unit dry mass, but per unit shoot water when correlations with the consequences of ion accumulation are being sought. Both Na^+ and Cl^- concentrations increase in the shoots following exposure of the roots to these ions with, once a critical concentration is reached, a consequent reduction in vegetative growth. As ions are also part of the solutes that bring about osmotic adjustment, studies on their compartmentation in leaf cells are required. For Cl^- there is the important and confounding factor that a significant proportion of any measured ions will be present

in secretions from trichomes on the leaf surface rather than within leaf cells. From the studies to date, the roles of ion selectivity by roots and the ion, sugar and compatible solute accumulation and osmotic adjustment by shoots in salt resistance/sensitivity among genotypes are not clear; studies of differences in accumulation of ions and sugars between salt-sensitive and salt-resistant genotypes are warranted.

N_2-FIXATION

Salt tolerance of chickpea rhizobia

Chickpea is clearly a very salt-sensitive species, the most sensitive genotypes failing to survive in 25 mM NaCl and the most resistant being unlikely to survive in 100 mM NaCl in hydroponics (see preceding section on vegetative growth). By contrast, rhizobia isolated from chickpea nodules and cultured *in vitro* are typically much more salt tolerant, with some strains able to grow in 500 mM NaCl (Kucuk & Kivanc 2008). Strains do, however, differ in NaCl tolerance: while some strains can grow at salt concentrations as high as 500 mM, others will not grow at 300 mM and a few will not even grow at 100 mM NaCl (e.g. Elsheikh & Wood 1990b; Zurayk *et al*. 1998; Kucuk & Kivanc 2008). Differences in salt tolerance amongst 18 chickpea rhizobial strains were also evident at 20 dS m^{-1} (1:1 NaCl : $NaSO_4$), with lag phases for growth ranging from 34 to 154 h and growth either completely inhibited or not reduced relative to non-saline controls (Zurayk *et al*. 1998). Nevertheless, tolerance appears to be relatively high amongst rhizobia isolated from diverse sources of chickpea nodules; except for one strain (N7 in Kucuk & Kivanc 2008) all the rhizobia show 'free-living' growth at NaCl concentrations exceeding that resisted by chickpea plants (Elsheikh & Wood 1990b).

Despite the apparent tolerance of 'free-living' chickpea rhizobia, salinity can have more adverse effects on nodulation and N_2-fixation than on overall growth of chickpea, as evidenced by comparisons of nodulated and mineral N-fed plants under saline treatments (e.g. 75 mM NaCl Lauter *et al.* 1981, 3 dS m^{-1} Zurayk *et al*. 1998). Attention to the effects of salinity on the chickpea–rhizobium symbiosis is clearly warranted and explored in the following discussion.

Nodulation

Several studies have demonstrated decreased nodulation of chickpea under saline conditions. Even at just 1 dS m^{-1} (a mixture of NaCl and $CaCl_2$ to give 4:1 $Na^+ : Ca^{2+}$ when added to soil), nodule numbers were already reduced to 85% of controls (Elsheikh & Wood 1990a) and substantial reductions (e.g. to 35–58% of controls) occurred at 3 to 4 dS m^{-1} (Sekhon *et al*. 1987; Elsheikh & Wood 1990a; Zurayk *et al*. 1998; Rao *et al*. 2002). Two studies, however, indicate that nodulation need not be the most salt-sensitive component of chickpea performance in saline conditions. The cultivar Pusa-312 inoculated with strain P-114-3 showed no effect of NaCl at 4 dS m^{-1} and even at ~8 dS m^{-1} only a modest decrease on nodule numbers (83% of control, Saxena & Rewari 1991). Similarly, whereas 8 dS m^{-1} decreased nodule numbers in two 'salt-sensitive' genotypes to ~75% of the control, there was no decrease for two 'salt-resistant' genotypes (Garg & Singla 2004). In both these studies, salinity treatments were severe enough to reduce plant growth and were applied at sowing (some other studies, not considered in this section on nodulation, imposed salinity treatments after plant establishment and are not discussed here as nodulation could have occurred prior to addition of salinity). As discussed in the next paragraph, differences in sensitivity of nodulation of chickpea to salinity reported in various studies, might be explained by differences in resistance of the host plant and specific interactions between host and bacterial strain (i.e. resistance of the symbiosis).

Different strain × host combinations result in vastly different nodulation success under saline conditions, as demonstrated for eight rhizobial strains and five chickpea cultivars in a saline field with an EC (presumably ECe) of 4.5–5.2 dS m^{-1} (on a research farm near New Delhi, with two flood irrigations applied; dominant ions in soil or water not specified, Saxena & Rewari (1992). Such observations have lead to the view that improving salt resistance in field-grown chickpea requires selection of salt-resistant chickpea cultivars in combination with rhizobial strains chosen for their capacity to enter an effective symbiosis for nodulation and N_2-fixation under saline conditions (Saxena & Rewari 1992). Both plant genotype (Tejera *et al*. 2006) and rhizobial strain (Lauter *et al*. 1981; Rao & Sharma 1995a) determine the degree of nodulation, so plant interactions should be evaluated under saline conditions and the best combinations selected (Saxena & Rewari 1992). Adding to this complexity, symbioses can differ in sensitivity to different types of salts; a salt-mix with Cl^- as the dominant anion has been reported more detrimental than one dominated by SO_4^{2-}, but this study only evaluated one genotype and one strain (Kumar & Promila 1983). An alternative approach, with implications of easier selection screens if shown to be appropriate, was proposed by Rao & Sharma (1995a). They suggested that as the most effective strains under control conditions also produce most nodules under saline conditions, selection could be made for nodulation effectiveness *per se*. This approach should, however, be applied to salt-resistant chickpea lines. Performance in saline fields depends upon salt resistance of the chickpea plant, as well as having suitable rhizobia for a robust symbiosis under saline conditions and some large-scale screening programmes for salt resistance in chickpea have included nodule numbers and activity as priority traits (Sadiki & Rabih 2001), although it has not been reported whether this approach resulted in improved cultivars being released for salt-affected soils.

Nodule growth and senescence

Impeded nodule growth, resulting in smaller nodules (e.g. fig. 2A in Elsheikh & Wood 1990a), as well as decreased

nodule numbers (discussed above), can together decrease the total nodule biomass present in salt-affected chickpea. Average nodule mass, calculated from nodule numbers and total mass, shows nodule size can be decreased by salinity and smaller nodules occur even in situations where nodule numbers did not decline (e.g. calculated from Saxena & Rewari 1992). A useful parameter to consider is the ratio of nodule mass to root mass, as this should indicate sensitivity of nodules relative to the roots. Data from the few studies available show divergent responses in nodule mass : root mass ratio to salinity. Substantial declines (e.g. Rao *et al*. 2002) indicating nodules as being more salt sensitive than the host roots as well as increases in this ratio (Garg & Singla 2004), indicating nodulation and nodule growth as being less sensitive than the roots have been reported. In addition to the effects of salinity on nodulation and nodule growth, NaCl can also promote senescence (assessed as declines in leghemoglobin concentration) of chickpea nodules (Sheokand, Dhandi & Swaraj 1995; Nandwal *et al*. 2007), which would also contribute to declines in the numbers and mass of functional nodules and thus N_2-fixation under saline conditions.

Nodule ion concentrations and osmotic relations

To our knowledge, only one study has reliably assessed ion concentrations in nodules of chickpea under saline conditions (Sharma & Kumar 1992). A recent paper (Nandwal *et al*. 2007) reported Na^+/K^+ ratios in nodules, but these data should be considered with extreme caution as non-saline controls were reported to contain 28-fold more Na^+ than K^+. As no data for tissue Na^+ and K^+ concentrations were presented in Nandwal *et al*. (2007), the reason for this discrepancy is unknown (only nodule tissue Cl^- concentrations were presented).

In the experiment conducted by Sharma & Kumar (1992), two cultivars (BG-312 and Pusa-209) were raised for 1 month under non-saline conditions and then exposed to 4 or 8 dS m^{-1} for 20 d (salt mixture, $Na^+ : Ca^{2+} : Mg^{2+}$, 5:2:3; $Cl^- : SO_4^{2-}$, 7:1). K^+/Na^+ ratio declined in all tissues, but it was maintained best in nodules. At 8 dS m^{-1}, nodule K^+/Na^+ ratio decreased from 12.9 to 4.8 in Pusa-209, but did not decline from the initial 9.7 in the more 'salt resistant' BG-312. By contrast, in roots, the decline in K^+/Na^+ ratio was from 6.3 in Pusa-209 and 4.7 in BG-312, to 0.5 in both genotypes; and in leaves from 37 to 1.4 in Pusa-209 and 38 to 1.2 in BG-312. Tissue concentrations on a water basis for Na^+ and Cl^- in nodules were, respectively, ~40 mM and ~50 mM in BG-312 and ~65 and 100 mM in Pusa-209. Interestingly, K^+ increased markedly in nodules in response to salinity in both genotypes, reaching ~210 mM in the 'more resistant' BG 312 and went even higher to ~310 mM in the 'less resistant' Pusa-209. By contrast with nodules and in both genotypes, root Na^+ concentrations (up to ~100 mM) were substantially higher whereas those of K^+ (~90 to 100 mM) were significantly lower. In a study to evaluate the influence of exogenous K^+ under saline conditions (4.3 or 8.3 dS m^{-1} NaCl) nodule number was increased when K^+ was sufficient, but average nodule mass did not increase when K^+ supply was increased (calculated from Saxena & Rewari 1993).

Preferential allocation of K^+ to nodules at concentrations reaching 200 to 300 mM on a tissue water basis (Sharma & Kumar 1992) would contribute to osmotic adjustment of these tissues and might also reflect a higher cytoplasmic to vacuolar ratio in nodules compared with whole roots. There is a limit, however, to the levels of K^+ that can be accumulated in the cytoplasm, as high K^+ will also inhibit enzymes (Greenway & Osmond 1972). In addition to these large increases in K^+ in nodules (Sharma & Kumar 1992), total soluble sugars and proline (see Table S2) also increased under saline conditions and these might contribute to osmotic adjustment (Soussi *et al*. 1998, 1999; Nandwal *et al*. 2007), although increases in these organic solutes can be modest compared with changes in osmotic potential in the nodules (e.g. from –0.75 MPa in controls to –1.77 MPa at 10 dS m^{-1}, Nandwal *et al*. (2007). Moreover, accumulation of sugars and proline were higher in nodules of a 'salt-sensitive' (Pedrosillano) than a 'salt-resistant' (ILC-1919) cultivar (Soussi *et al*. 1999).

N_2 fixation capacity

The capacity for N_2-fixation by chickpea in saline soils can decline owing to the inhibitory effect of salinity leading to fewer nodules, which can typically be smaller (discussed above, see also Babber, Sheokand & Malik 2000) than those produced in the absence of salt. In addition, N_2-fixation capacity per unit nodule mass may be reduced under saline conditions. Several studies have used an acetylene reduction assay to evaluate the potential capacity for N_2-fixation of chickpea nodules under various salinity regimes. The data show large inhibitions of N_2-fixation capacity with increasing salinity. For example, for detached nodules from plants exposed to 2.5 dS m^{-1} ($NaCl : Na_2SO_4 : CaCl_2$, 1:0.6:0.3) in soil without applied mineral N, acetylene reduction was 36% of the non-saline control (Sekhon *et al*. 1987). In other cases, the decline in acetylene reduction was more moderate; for example, at 50 mM NaCl applied to chickpea in sand culture, acetylene reduction by nodules was ~70% of the control, but at 100 mM the reduction was again severe so that the rate was only 20% of the control (Sheokand *et al*. 1995). We hypothesize that the difference in the degree of inhibition that can occur at moderate salinity (36% of control at 2.5 dS m^{-1} versus 70% of control at 50 mM, in the two studies discussed immediately above) presumably reflects differences in resistance of the symbioses, since an experiment with 10 chickpea cultivars inoculated with one rhizobial strain showed a wide range of inhibitions by 100 mM NaCl; acetylene reduction in the saline treatment ranged from 11 to 77% of the control values (Singh *et al*. 2005).

The causes for salinity-induced reductions in N_2-fixation capacity in chickpea nodules has been considered in a number of studies. These have focused on salt-induced

changes in nodule physiology and are discussed in the next paragraph. Firstly, we consider cause and effect; does inhibited N_2-fixation capacity lead to reduced tissue N and therefore growth reductions, or does impeded growth or reduced carbon availability from photosynthesis result in decreased N demand and therefore a decline in nodule activity? That reduced N_2-fixation capacity might lead to reduced N nutrition for the host plant is suggested by a decline in nodule N concentration to 40–50% of the control (Sheokand *et al*. 1995). However, in the experiments on acetylene reduction, the only studies that also measured shoot N concentrations showed that despite substantial reductions in nodule activity, shoot N concentrations hardly decreased (92–96% of control, Saxena & Rewari 1991) or even increased (102–110% of control, Saxena & Rewari 1991). The proportion of shoot N derived from N_2-fixation was maintained at salinities up to 6.2 dS m^{-1} in both salt-sensitive (CSG-8890) and resistant (CSG-8927) genotypes (Rao *et al*. 2002). Moreover, in a saline field, cultivar + strain combinations with best nodulation did not necessarily produce the highest yields (cf. figure 1A,B in Saxena & Rewari 1992). Thus, it is our opinion that while it is clearly desirable to have the best possible cultivar + strain combinations, additional evidence is required to support the notion that limited N nutrition contributes to poor chickpea performance in saline fields.

Nodules showing decreased acetylene reduction capacity and by inference a decline in nitrogenase enzyme activity per unit nodule mass, also displayed other salt-induced lesions. Leghaemoglobin concentration in nodules from plants at 50 mM NaCl declined to 59% of that in controls and was only 7% at 100 mM (Sheokand *et al*. 1995). These substantial reductions at 100 mM NaCl, a level of salinity that can kill many chickpea cultivars (see the section on vegetative growth) were also observed by Kumar & Promila (1983) and in other studies have been interpreted as nodule 'senescence' (Babber *et al*. 2000). It is our view that these responses to high salinity appear to be salt-induced death/necrosis, rather than 'senescence'.

Prior to 'senescence' or necrosis, other factors could also impede N_2-fixation activity in nodules. Photosynthate supply should not, however, restrict N_2-fixation in saline conditions, as sugars and amino acids accumulate in nodules of chickpea (Soussi *et al*. 1998). Soussi *et al*. (1999) proposed that an inhibited conversion of sugars to malate (regarded as the preferred substrate for the bacteroids, Kim & Copeland (1996) in chickpea nodules, could restrict N_2 fixation under saline conditions. At 50 mM NaCl, malate concentration in nodules of 'salt-sensitive' Pedrosillano was 23% of the control, whereas in the 'salt-resistant' ILC-1919 it was 56% of control (Soussi *et al*. 1999). This view of malate limitation was, however, not supported by subsequent experiments by the same group showing that chickpea bacteroid respiration is supported by a range of substrates (Soussi *et al*. 2001), so that malate was presumably not limiting nodule activity.

Lower nodule gas permeability can decrease N_2-fixation rates (Hunt & Layzell 1993). Nodule O_2 conductance can be regulated by physiological modulation of the O_2-diffusion barrier in nodules and/or by structural changes. Structural changes in salt-affected nodules of chickpea might reduce nodule conductance to O_2 and restrict N_2-fixation capacity (Babber *et al*. 2000). However, the only attempt to measure chickpea nodule O_2 permeability did not separate nodules and roots (L'Taief *et al.* 2007) and since nodules are only a small fraction of the root mass (viz. less than 10%) the O_2 consumption by roots would have presumably dominated the measurements and, therefore, any changes in nodules might not have been detected. Increased activity of alcohol dehydrogenase in salt-exposed nodules of chickpea might indicate a change in O_2 supply (Soussi *et al*. 1999), so additional work on this topic seems warranted.

In summary, chickpea rhizobia isolated from diverse sources show a higher 'free-living' salt tolerance (up to 500 mM NaCl) than chickpea plants (up to about 100 mM NaCl). Despite the high apparent salt tolerance of the rhizobia, salinity can cause large reductions in nodulation, nodule size, N_2-fixation capacity and can, in severe cases, cause nodule necrosis. Considerable variation exists amongst chickpea genotype × rhizobia strain combinations in nodule numbers (Saxena & Rewari 1992) and functioning (Singh *et al*. 2005) in saline conditions. Thus, breeding of improved chickpea genotypes for salinity resistance should also include evaluations during the breeding process with a range of rhizobial strains under salt-affected conditions.

REPRODUCTIVE GROWTH AND GRAIN YIELD

Seed yield is affected by the number of pods and/or the number of seeds per pod; the mass of individual seeds might also be reduced where plant growth is reduced. Pod number is a function of the number of flowers and their successful pollination. Flower number has been shown to be differentially affected across genotypes under saline conditions as was pollen production (Dhingra & Varghese 1993). Dhingra & Varghese (1993) found a toxic effect of SO_4^{2-} at 60 mM on pollen tube growth, but not on pollen germination. Three genotypes exposed to 60 mM of mixed salts suffered reduced pollen production per flower to 48–55% of controls (Dhingra *et al*. 1996). Pollen germination was not affected by 32 mM NaCl, whereas the same concentration of Na_2SO_4 reduced germination by as much as 50% in one genotype. Pollen tube length was, however, impeded by 32 mM of either salt; lengths were reduced to 50% in NaCl and to 25% in $NaSO_4$ (Dhingra & Varghese 1993). Any reduction in yield per plant could be brought about by a decrease in the number of flowers per plant and/or their effectiveness in producing seed, determined by the viability of the pollen and the receptivity of the stigmas, and photosynthates available for seed filling.

Datta, Dayal & Goswami (1987) reported that the resistant line H-82-2 produced more flowers than sensitive lines and that the percentage of abscission of flowers across lines did not differ between genotypes. This agrees well with current observations of a higher number of flowers in

resistant than sensitive lines under control conditions (V Vadez, unpublished results 2009). Similarly, the higher salt resistance of a late-maturity variety was attributed to it producing a larger number of flowers than an early-maturing line (Katerji *et al*. 2001). Salinity has been shown to decrease the number of pods per plant, the number of seeds per pod and the individual size of seeds (Mamo *et al*. 1996; Katerji *et al*. 2001). The seeds produced under saline conditions can be shrivelled and with a reduced protein concentration (Kumar *et al*. 1983), although seed mass may only be reduced by 10% (Dua 1992) or 20% (Vadez *et al*. 2007). Dua (1992) reported that although the 1000-seed mass was reduced by 10%, this was less affected than other yield components such as number of pods and number of seeds (reduced to 38% and 33%, respectively). Similar relative sensitivities of the yield components to salinity were found by Vadez *et al*. (2007) who reported that while 1000-seed mass was reduced by 20%, the seed number was reduced about 50%. So in summary, it appears that reproduction is a sensitive developmental stage for chickpea exposed to salinity. Producing a larger number of reproductive structures in the absence of salt may be a simple constitutive trait contributing to the maintenance of seed or pods/plant.

Few studies have compared the salt resistance of chickpea at different growth stages. One notable exception was an evaluation of six lines for salt resistance when grown to maturity; three lines were selected at the seedling stage as 'resistant' and three others as 'sensitive' (Ashraf & Waheed 1993). Only two of the resistant lines and one sensitive line performed as expected to maturity in soil salinized with 40 mM NaCl; that is, one 'resistant' line failed to produce any seed as it died, whereas two 'sensitive' lines survived and produced some seed. These results show that selections for salt resistance are required across the entire life cycle and genotypes differ in expression of resistance at different stages, presenting an opportunity to combine sources of resistance for difference stages from contrasting parents. In this study, seed yield at 40 mM NaCl ranged from 0 to 23% of non-saline controls (Ashraf & Waheed 1993) and the effect on yield parameters also differed between genotypes. For the two most resistant genotypes, CM-663 retained pods at 70% of the control and ICC-10572 at 50%, whereas CM-663 suffered reductions in 1000-seed mass (seed size) to 40% of the control but 1000-seed mass did not decrease in ICC-10572. By contrast, Datta *et al*. (1987) reported no significant difference amongst five 'salt resistant' genotypes in percentage of flowers that developed into pods under saline conditions.

Ion relations

Although studies have shown shoot dry mass at the flowering stage and seed yield at maturity were negatively correlated to shoot Na^+ concentrations (Manchanda & Sharma 1989), others have found no correlation of seed yield and shoot mass at flowering under salinity and have therefore suggested that differences in resistance at the reproductive stage determine salt resistance in chickpea (Vadez *et al*. 2007). Only two studies (Table 6) have evaluated ion concentrations in reproductive structures of salinized chickpea. When chickpea (cv. Chafa) was grown at 50 mM NaCl in sand culture, pod wall Na^+ and Cl^- were, respectively, 720 and 1420 μmol g^{-1} dry mass and seed Na^+ and Cl^- were, respectively, 420 and 870 μmol g^{-1} dry mass (Murumkar & Chavan 1986), demonstrating that potentially-toxic levels of ions might occur in both these tissues. These high ion concentrations occurred despite the pots being flushed in alternate irrigations with saline and fresh solutions, a practice that prevents a steady state from occurring. By contrast, K^+ concentration was not affected in seeds or in pod walls, whereas Ca^{2+} decreased markedly in pod walls but not in seeds. In four genotypes grown at 2 dS m^{-1} NaCl, seed Cl^-

Table 6. Ion concentrations observed in reproductive structures of various chickpea genotypes

			Ion concentration (μmol g^{-1} dry mass)				
Genotype	Treatment NaCl	Plant part	Na^+	Cl^-	K^+	K^+/Na^+ ratio	Source
Chafa	0	Grain	140	460	440	3.1	Murumkar & Chavan
Chafa	50 mM	Grain	420	870	400	1.0	(1986)
Chafa	100 mM	Grain	520	870	430	0.8	
Chafa	0	Pod wall	100	330	1360	13.6	
Chafa	50 mM	Pod wall	720	1420	1430	2.0	
Chafa	100 mM	Pod wall	2390	2240	1360	0.6	
DZ-local	0	Grain	203	68	682	3.4	Mamo *et al*. (1996)
DZ-local	20 mM[a]	Grain	365	383	686	1.9	
Mariye	0	Grain	230	67	657	2.9	
Mariye	20 mM[a]	Grain	365	na	na	na	
DZ-10-9-2	0	Grain	223	86	693	3.1	
DZ-10-9-2	20 mM[a]	Grain	298	350	658	2.2	
DZ-10-16-2	0	Grain	227	85	700	3.1	
DZ-10-16-2	20 mM[a]	Grain	317	353	691	2.2	

[a]2 dS m^{-1}.
na, data not available.

concentration increased on average 4.6-fold and Na^+ 1.5-fold, compared with non-saline controls, but K^+ was not affected (Mamo *et al*. 1996). The level of Cl^- in the seeds was, on average, 15% of that in the vegetative tissues of the shoot (Mamo *et al*. 1996). Shoot tissue Na^+ was surprisingly low by comparison with Cl^- (shoot Cl^- : Na^+ was 4.5 on a molar basis) and so the seed Na^+ concentration was approximately equal with that in the vegetative tissues (Mamo *et al*. 1996).

Water relations

As far as is known, the water relations of pods of chickpea subjected to salinity have not been measured, but we speculate that a decrease in water potential of the leaves arising from salinity in the soil will induce a decrease in the water potential of the pod wall, but may not influence the seed itself. This is based on observations by Shackel & Turner (2000) that when chickpea was subjected to a water shortage, the water potential of the pod was the same as that of the leaf when both were covered to prevent differences in transpiration. However, when the water potential of the pod fell from –0.37 to –1.22 MPa, the turgor pressure of the cells in the pod wall decreased from 0.97 to 0.25 MPa, while the turgor pressure of the seed coat remained constant at 0.11 MPa (Shackel & Turner 2000), suggesting that the seed is buffered from the decrease in water potential and water content in the plant and even in the pod wall. As in leaves (see preceding section on water relations), the proline concentration in pod walls can double and the concentration in seeds increase by 40% when exposed to 100 mM NaCl (Murumkar & Chavan 1986, Table S2). Apart from the effects on the water relations of the pod, water shortage has been shown to decrease pollen and stigma viability and increase flower and pod abortion (Leport *et al.* 2006; Fang *et al.* 2009), so that the decrease in water relations under salinity may also influence pollen viability and flower and pod abortion before any ionic imbalances begin to play a role in the reproductive processes, but this need to be evaluated in future studies.

In summary, the few data available indicate differences in salt sensitivity and resistance amongst chickpea genotypes at the reproductive phase. The physiological basis of these differences is uncertain. Na^+ and Cl^- concentrations in reproductive structures have rarely been measured, but the available data indicate that concentrations can be relatively high (Table 6), so that the possible toxicity of these ions, as well as changes in hormonal status or water relations should all be evaluated in future research. Yield experiments are best conducted in saline fields, even though salinity is very variable over short distances (Bennett, Barrett-Lennard & Colmer 2009), so that large plots and/or numbers of replicates are needed to improve the precision of field-based screens, and data on the conditions across sites used should be recorded, which limits the number of accessions that can be tested.

GENETICS AND PLANT IMPROVEMENT

Approaches to improving the yield of chickpea in salt-affected soils

Genetic variability is a prerequisite in breeding for improvement of any trait and efforts have been made to assess genetic diversity in chickpea germplasm for salinity resistance. Various traits have been used in screening for resistance to salinity including: germination percentage, radicle length, shoot length, nodulation, leaf necrosis, salinity susceptibility index (based on biomass yield under saline and non-saline conditions), plant biomass, number of pods per plant and grain yield (Table 7). As the ultimate criterion for salinity resistance is the grain yield under saline conditions, the traits/indices used for assessing salinity resistance must be correlated with grain yield.

Limited efforts have been made in breeding for salt resistance in chickpea (e.g. Dua 1998; Dua & Sharma 1995, 1997; Dua *et al*. 2000). As discussed below, only 4 of the 13 studies listed in Table 7 have evaluated grain yield under saline conditions and identified salinity resistant genotypes (Singh *et al*. 1994; Dua & Sharma 1995, 1997; Sharma, Singh & Dua 2004; Vadez *et al*. 2007).

Low variability among genotypes for salinity resistance, as indicated by early studies based on plant growth in pots (Johansen *et al*. 1990), was considered a major bottleneck in improving salinity resistance in chickpea. However, recent screenings of relatively large numbers of diverse genotypes suggest wide variation for salinity resistance based on seed yield under controlled saline conditions (Vadez *et al*. 2007, see also Table 8 and Tables S3 & 4). A screening of 263 accessions, including 211 accessions from ICRISAT's mini-core collection (10% of the core collection and 1% of the entire collection), at ICRISAT-Patancheru (Vadez *et al*. 2007) showed a six-fold range of variation for seed yield under salinity, with several genotypes yielding 20% more than a previously-released salinity resistant cultivar (Karnal Chana 1 or CSG-8962), The range of variation in yields under salinity was similar in both *kabuli* and *desi* chickpeas, indicating that breeding for salinity resistance can be undertaken in both types. Based on grain yield under saline conditions (80 mM NaCl in pots of soil, Vadez *et al*. 2007) found that *desi* types are more resistant to salinity than *kabuli* types, although the opposite was found by Dua & Sharma (1995). Other studies (Sinhgla & Garg 2005; Sohrabi, Heidari & Esmailpoor 2008), based on screening of few genotypes, support the findings of Dua & Sharma (1995). Germplasm from regions of the Middle East and South Asia, where there has been a history of soil salinity, revealed the most diversity for salinity resistance (Maliro *et al*. 2008).

No relationship has been found between salinity resistance at germination and maturity (Dua & Sharma 1995). One study (Manchanda & Sharma 1990) found a good agreement between seedling stage evaluation and yield-based assessment in chickpea (Mamo *et al*. 1996), although this experiment was carried out with only four genotypes. In another study, for some genotypes (ICC-10130, ICC-10582, ICC-12909) there was a negative correlation for salt

Table 7. Some examples of the identification of genetic variability and salinity resistance in chickpea

NaCl concentration	Genotypes screened (Growth media)	Resistant genotypes identified	Traits studied to asses salt resistance	Source
50 mM	160 (Nutrient solution)	L-550	Percent survival at 9 weeks after planting	Lauter & Munns (1986a)
1, 2, 3, 5 dS m^{-1}	81 (Soil pots)	No substantial variation of salinity tolerance was observed	Reduction in shoot growth	Johansen *et al.* (1990)
0.5, 1, 1.5, 2%	10 (In vitro, Petri dishes)	Pusa-312, Pusa-212, Pusa-240	Germination % & radicle length	Saxena & Rewari (1991)
2, 4, 6, 8 dS m^{-1}	395 (Field)	CSG-88101, CSG-8927, CSG-8977, CSG-8962, CSG-8943 (resistance based on yield) CSG-8893, CSG-8965, CSG-8916, CSG-8922, CSG-8966 (resistance based on germination %)	Germination % & seed yield	Dua (1992), Dua & Sharma (1995), Dua & Sharma (1997)
8 dS m^{-1}	58 (Hoagland Solution)	JCP 27, K 850, AKG 46, DGM 1044, JCP 125	Germination & seedling growth	Kathira *et al.* (1997)
25, 50 mM	200 (Sand)	MCA-31, MCA-45, MCA-103, MCA-131, MCA-250	Nodule mass & plant mass	Sadiki & Rabih (2001)
0.5, 4, 7, 10 dS m^{-1}	205 (In vitro, Petri dishes)	FILIP-87-59, IFLIP-97-158, FILIP-97-205, FILIP-97-259, FILIP-98-128	Germination & shoot length	Karajeh *et al.* (2003)
4, 6, 8 dS m^{-1}	30 (In vitro, Petri dishes & sand)	GSI > 60 at 8 dS/m (C-14.5.85, C-1.9.85, C-6.9.85, C-9.9.85, C-13.9.85 DMSI > 20 (C-17.4.85, C-5.9.85, C-20.9.85, C-21.9.85, CLN-86)	Germination stress index (GSI), seedling height stress index (SHSI) & Dry matter stress index (DMSI)	Al-Mutata (2003)
100 mM	252 (Soil pots)	ICC-10755, ICC-13124, ICC-13357, ICC-15406, ICC-15697	Salinity susceptibility index (SSI) & shoot biomass	Serraj *et al.* (2004)
5.4 dS m^{-1}	83 (Field condition)	CSG-9546, CSG-8962, SG 90-G9-1, SG 96-R-3, SG 96-GII,	Pods per plant, seeds per plant and seed yield per plant	Sharma *et al.* (2004)
0, 4, 6, 8 dS m^{-1}	8 (Sand)	KC-1, HC-3, C-235	chlorophyll stability index (CSI), K/Na ratio and seed yield	Asha & Dhingra (2007)
80 mM	263 (Soil pots)	ICC-1431, ICC-15610, ICC-5003, ICC-4593, ICC-12155	Seed yield	Vadez *et al.* (2007)
6 dS m^{-1}	200 (Sand)	ICC-30, ICC-8980, ICC-903, ICC-801, ICC-6671	Leaf necrosis & biomass	Maliro *et al.* (2008)

Table 8. Effects of salinity on yield of selected genotypes of chickpea. Salt treatments were applied from the time of sowing and biomass data collected at maturity

Relative yield, genotype and reputation (% of controls)			
Sensitive	Resistant	Salt treatment	Source
55% ICC6263	74% ICC-1431	1.17 g NaCl kg^{-1} soil in a Vertisol (80 mM NaCl)	Vadez *et al*. (2007)
0% ICC-12908	20% CM-663 14% ICC-10572	40 mM NaCl in hydroponic sand culture	Ashraf & Waheed (1993)
57% Filip-87-59c	98% ILC-3279	EC_e of 2.5 dS m^{-1} in soil salinized with NaCl and $CaCl_2$	Katerji *et al*. (2005)
27% DZ-Local	53% DZ10-16-2	0.62 g NaCl kg^{-1} soil	Mamo *et al*. (1996)

resistance between early growth stages and adult stage (Ashraf & Waheed 1992, 1993). Serraj *et al*. (2004) and Vadez *et al*. (2007) screened the mini-core collection of chickpea at ICRISAT under saline conditions for plant biomass and seed yield, although none of the genotypes identified to be resistant in these studies was common between the two studies. Vadez *et al*. (2007) grew two sets of 263 chickpea genotypes simultaneously. One set was harvested at 40 DAS while the other set was harvested at maturity. Data that showed the biomass under salt stress or biomass under salt stress relative to control was not related to either seed yield under salt stress or to the seed yield under salt stress relative to control. This emphasizes the fact that the final assessment of salinity resistance should be based on grain yield. Thus, the salinity-resistant genotypes identified based on traits other than seed yield and its component traits (e.g. number of pods per plant) in different studies (Saxena & Rewari 1992; Kathira, Nayagapara & Vaddoria 1997; Al-Mutata 2003; Karajeh *et al*. 2003; Serraj *et al*. 2004; Maliro *et al*. 2008) need further confirmation by evaluating these genotypes for seed yield under saline conditions.

There are several factors that reduce the efficiency of conventional breeding approaches for salt resistance where grain yield under salinity is used as the criterion for salt selection. These include limited genetic variability for salt resistance, inadequate screening techniques for segregating generations, limited knowledge of the genetics of resistance, complexity of the several resistance mechanisms involved and poor understanding of salinity and environmental interactions in a highly variable environment (cf. Flowers & Yeo 1986). The heterogeneity for salinity in fields and the large genotype × environment interaction for salt resistance necessitates screening of test materials over years and locations using a large number of replications in each trial. This makes it difficult to select for salt resistance in segregating populations. As a consequence, as far as we are aware, only one salt-resistant cultivar of *desi* chickpea, Karnal Chana 1 (CSG-8962) has been released for north-western parts of India (Dua *et al*. 2000). This variety can be grown in saline soils with an ECe between 4 to 6 dS m^{-1} (Dua *et al*. 2000). Considerable further effort is required if new salt-resistant genotypes are to be developed.

A strong relationship ($R^2 = 0.50$) between seed yield under salinity and seed yield under a non-saline control, suggests that a yield potential component as well as salinity resistance *per se* (Vadez *et al*. 2007) is important for any breeding programme. This suggests that available high yielding cultivars may provide the basis for identifying cultivars that can perform well in saline soils. If so, genotypes that perform well in non-saline conditions might perform relatively better than other lines in saline fields (c.f. Richards 1983). As seed size was similar in the salt-resistant and salt-sensitive genotypes identified by Vadez *et al*. (2007), it should be possible to develop salt-resistant cultivars in the market-preferred seed size category.

Apart from breeding for yield *per se*, the possibility remains to identify traits important in resistance and pool these traits as suggested for another salt-sensitive species, rice (Yeo *et al*. 1990). The use of such an approach is, however, highly labour intensive and requires collaboration of physiologists and breeders and that selections are not made early in the breeding process (Garcia *et al*. 1995). For chickpea, in spite of its importance as a food crop, there is a dearth of physiological information on traits for salt resistance. Examples of important questions for which we have no answer at present are: can selection for transport of Na^+, K^+ and/or Cl^- be used to improve the overall salt resistance and does tissue tolerance of these ions vary between genotypes? Molecular markers linked to genes/ quantitative trait loci (QTLs) controlling salt resistance and/or the component traits for salt resistance, if available and robust, would greatly facilitate selection of resistant plants in segregating generations and help accelerate genetic advance. As salt resistance is controlled by many genes, marker-assisted recurrent selection (MARS) might also be used to accumulate salt resistance genes from different genotypes. MARS is an approach in which progenies are selected for intercrossing using a selection index constructed based on QTL-associated molecular markers (Charmet *et al*. 1999, 2001).

Genetics of salinity resistance

Plant response to salt stress is influenced by various physiological and agronomic characteristics, which may be controlled by the actions of several to many genes, whose expressions are influenced by various environmental factors (Foolad 2004). Thus, interactions between genotype

and environment need to be considered in identifying salt-resistant genotypes for breeding programmes.

In a study conducted by Ashraf & Waheed (1998) to estimate gene action for salinity resistance in chickpea, the parental genotypes included two resistant lines (CM-663, ICC-10572) and four sensitive lines (ICC-10130, ICC-10582, ICC-12908 and ICC-12909). Salt resistance was assessed by comparing seed yield per plant, number of pods per plant and number of seeds per pod under control and saline (40 mM NaCl) conditions. The authors concluded that salt resistance was governed by both dominance and additive gene effects with a preponderance of dominance effects. Parents with contrasting salt resistance had unequal gene distribution with excess of recessive alleles accumulated in resistant genotypes and dominant alleles in sensitive genotype, although the numbers of alleles were not specified. Heritability estimates (both broad- and narrow-sense) were high for all the three traits, suggesting that selection for these traits would be effective (Ashraf & Waheed 1998).

The expression of salt resistance is quantitative in nature and controlled by several genes or QTLs. While structural genomics approaches are used to identify QTLs controlling a trait, functional genomics approaches can unravel the genes involved (Varshney, Graner & Sorrells 2005). Structural genomics approaches include linkage mapping and association genetics for trait mapping using bi-parental segregating populations (e.g. F_2, double haploids, recombinant inbred lines) and natural populations, respectively. Molecular-markers associated with salinity resistance QTLs identified through linkage or association mapping approaches should be useful for accelerating the breeding for salt resistance through marker-assisted selection (MAS). Although molecular markers for salt resistance have been identified in some crops, to the best of our knowledge, no report is available on mapping of salinity resistance QTLs in chickpea. This can be partly attributed to the unavailability of appropriate mapping populations and limited genomic resources (Varshney *et al*. 2007). Nevertheless, recently, a *kabuli* (ICCV-2) and a *desi* (JG-62) type that contrast for seed yield under saline conditions (41% yield reduction in JG-62 as compared with 72% in ICCV-2 at 80 mM NaCl in soil pots (Vadez *et al*. 2007) have been used as parents of a RIL mapping population to identify markers for QTLs associated with salinity resistance. The development of 1655 novel SSR markers from BAC-end sequences and SSR enriched library at ICRISAT in collaboration with University of California (Davis) and University of Frankfurt, respectively should accelerate the molecular mapping of salinity resistance in chickpea. Furthermore, two new RIL populations are being developed at ICRISAT (ICC-6263 x ICC-1431 and ICCV-2 x JG 11) that can be used for validation of markers identified from ICCV-2 x JG-62 RILs and identification of additional QTLs.

Functional genomics includes several parallel approaches and tools such as EST generation, transcript profiling and reverse/forward genetics (Coram *et al*. 2007). Progress using these tools has, unfortunately, been slow in chickpea although there have been significant advances in the last few years (Varshney *et al*. 2009a). For instance, a new set of 3798 ESTs from JG-11 (salt-resistant) and 4460 ESTs from ICCV-2 (salt-sensitive) genotypes were developed at ICRISAT, providing a set of 2595 salt responsive unigenes. *In silico* differential gene expression analysis in the two EST datasets identified about 20 candidate genes that are presently being validated using Northern hybridization (Varshney *et al*. 2009b). Using a pilot microarray (ca. 750 features) for transcript profiling, Mantri *et al*. (2007) identified 386 genes that showed more than two-fold differences in transcriptional changes in response to salinity in chickpea. In this set, 30 genes were consensually differentially expressed between resistant (CPI-060546, ICC-06474) and sensitive (CPI-60527, ICC-08161) chickpea genotypes. Similarly by using SuperSAGE technology, Molina *et al*. (2008) also identified several candidate salinity-responsive genes. A set of selected salinity responsive genes have been listed in Table S6. Candidate genes identified through *in silico* or microarray experiments can be used for mapping and identification of genes, if any, from the identified set that are associated with QTLs for salt resistance. Similarly, a TILLING population developed for ICC-4958 at ICRISAT, can also be used for validating the function of identified candidate genes through reverse genetics approaches.

CONCLUSIONS

Germination is, relative to vegetative growth, resistant to salinity in some, but not all, genotypes. Vegetative growth is very sensitive to salinity with dramatic reductions in dry matter production and yield occurring at salt concentrations of less than 80 mM NaCl or about 8 dS m^{-1}. The few data available suggest that if the effect of salinity on vegetative growth is assessed in hydroponics, soil in pots or in the field, the rank order of resistance of genotypes to salt is consistent. However, just why chickpea is so sensitive to salt is not clear. In some experiments, growth reduction appears to be related to an inability of chickpea plants to prevent high concentrations of saline ions reaching the leaves. Growth under salinity is reduced once concentrations of Na^+ and/or Cl^- reach a critical concentration in the shoots, although for Cl^- correlations with growth may be confounded by ion secretion from glandular hairs. In some experiments in saline soils, Na^+ and Cl^- concentrations do not appear to reach critical concentrations. Genotypes may also differ in their ability to retain water, with the possibility that salt-resistant lines are better able to maintain a higher shoot water content than are more sensitive lines when growing in saline conditions. Ions are presumably used for osmotic adjustment, but it is uncertain from the available data whether adjustment always matches the external change in water potential. As far as yield is concerned, reproduction appears a particularly sensitive stage, with recent screenings of diverse genotypes suggesting significant variation of seed yield under saline conditions. As genotypes that produce many pods under non-saline conditions are those that yield better under saline conditions, producing a large number of reproductive structures in the

absence of salt may be a simple constitutive trait contributing to the maintenance of seed or pods/plant under saline conditions. Limited data available indicate Na^+ and Cl^- concentrations can be relatively high in pods and seeds, so that their toxicity, as well as changes in hormonal status or water relations should be evaluated in future research.

Rhizobia have high apparent salt resistance, although salinity can cause large reductions in nodulation, nodule size, N_2-fixation capacity and even nodule necrosis. Considerable variation exists amongst chickpea genotype × rhizobial strain combinations in nodule numbers and functioning. Thus screening and breeding of improved chickpea genotypes should be evaluated using a range of strains of rhizobium under salt-affected conditions.

Although chickpea is a salt-sensitive species, there appears to be enough genetic variation to enable improvement in yield under saline conditions via breeding. Breeding programmes should aim to increase yields under mildly-saline conditions (e.g. EC_e up to 8 dS m^{-1} in a Vertisol, but less in lighter-textured soils), such as occur in rainfed crops with 'transient salinity' in sub-soils and in many irrigated areas. Selections for salt resistance are required across the entire life cycle with an emphasis on the use of field experiments where yield is determined with genotype × environment interactions being important. As rankings of genotypes for resistance can differ between germination, vegetative and reproductive stages, a pyramiding approach to combine these sources of resistance, as well as the possible combining of genes relevant within each stage, using molecular approaches such as marker assisted selection, should result in useful increases in resistance.

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REFERENCES

Al-Mutata M. (2003) Effect of salinity on germination and seedling growth of chickpea (*Cicer arietinum*) genotypes. *International Journal of Agriculture & Biology* **5,** 226–229.

Asha & Dhingra H.R. (2007) An integrated approach for screening of chickpea genotypes for salinity tolerance. *Indian Journal of Plant Physiology* **12,** 378–382.

Ashraf M. & Waheed A. (1992) Screening chickpea (*Cicer arietinum* L) for salt tolerance. *Tropenlandwirt* **93,** 45–55.

Ashraf M. & Waheed A. (1993) Responses of some genetically diverse lines of chick pea (*Cicer arietinum* L) to salt. *Plant and Soil* **154,** 257–266.

Ashraf M. & Waheed A. (1998) Components of genetic variation of salt tolerance in chickpea (*Cicer arietinum* L.). *Archives of Agronomy and Soil Science* **42,** 415–424.

Baalbaki R.Z., Zurayk R.A., Adlan M.A.M. & Saxena M.C. (2000) Effect of nitrogen source and salinity level on salt accumulation of two chickpea genotypes. *Journal of Plant Nutrition* **23,** 805–814.

Babber S., Sheokand S. & Malik S. (2000) Nodule structure and functioning in chickpea (Cicer arietinum) as affected by salt stress. *Biologia Plantarum* **43,** 269–273.

Beltagi M.S. (2008) Exogenous ascorbic acid (vitamin C) induced anabolic changes for salt tolerance in chick pea (*Cicer arietinum* L.) plants. *African Journal of Plant Science* **2,** 118–123.

Bennett S.J., Barrett-Lennard E.G. & Colmer T.D. (2009) Salinity and waterlogging as constraints to saltland pasture production: a review. *Agriculture, Ecosystems & Environment* **129,** 349–360.

Bernstein L. (1961) Osmotic adjustment of plants to saline media. I. Steady state. *American Journal of Botany* **48,** 909–918.

Chandra S. (1980) The effect of edaphic factors on chickpea. In *Proceedings of the International Workshop on Chickpea Improvement* (eds J.M. Green, Y.L. Nene & J.B. Smithson), pp. 97–105. ICRISAT, Patancheru, Andhra Pradesh, India ICRISAT, Patancheru, Andhra Pradesh, India.

Charmet G., Robert N., Perretant M.R., Gay G., Sourdille P., Groos C., Bernard S. & Bernard M. (1999) Marker-assisted recurrent selection for cumulating additive and interactive QTLs in recombinant inbred lines. *Theoretical and Applied Genetics* **99,** 1143–1148.

Charmet G., Robert N., Perretant M.R., Gay G., Sourdille P., Groos C., Bernard S. & Bernard M. (2001) Marker-assisted recurrent selection for cumulating QTLs for bread-making related traits. *Euphytica* **119,** 89–93.

Colorado P., Rodriguez A., Nicolas G. & Rodriguez D. (1994) Abscisic-acid and stress regulate gene-expression during germination of chickpea seeds – possible role of calcium. *Physiologia Plantarum* **91,** 461–467.

Colorado P., Nicolas G. & Rodriguez D. (1995) Convergent effects of stress and ABA on gene expression during germination of chick-pea seeds. *Journal of Plant Physiology* **146,** 535–540.

Coram T.E., Mantri N.L., Ford R. & Pang E.C.K. (2007) Functional genomics in chickpea: an emerging frontier for molecular-assisted breeding. *Functional Plant Biology* **34,** 861–873.

Dang Y.P., Dalal R.C., Mayer D.G., *et al.* (2008) High subsoil chloride concentrations reduce soil water extraction and crop yield on Vertisols in north-eastern Australia. *Australian Journal of Agricultural Research* **59,** 321–330.

Datta K.S. & Sharma K.D. (1990) Effect of chloride and sulphate types of salinity on characteristics of chlorophyll content, photosynthesis and respiration chickpea (*Cicer arietinum* L.). Biologia Plantarum (Prague) **32,** 391–395.

Datta K.S., Dayal J. & Goswami C.L. (1987) Effect of salinity on growth and yield attributes of chickpea (*Cicer arietinum* L). *Annals of Biology* **3,** 47–53.

Dhingra H.R. & Varghese T.M. (1993) Flowering and male reproductive functions of chickpea (*Cicer arietinum* L) genotypes as affected by salinity. *Biologia Plantarum* **35,** 447–452.

Dhingra H.R., Kajal N., Chhabra S. & Varghese T.M. (1996) Flowering pattern and yield correlation in gram (*Cicer arietinum*) under influence of salinity and growth-regulators. *Indian Journal of Agricultural Sciences* **66,** 230–233.

Dua R., Sharma S. & Singh S. (2000) Genetic options in managing salinity-alkalinity for pulse production. In *Advances in Management of Biotic and Abiotic Stresses in Crop Plants: Proceedings of National Symposium on Management of Biotic and Abiotic Stresses in Pulse Crops, 26–28, 1998, Kanpur* (eds M. Ali, *et al.*), pp. 43–48. Indian Society of Pulses Research and Development, Kanpur, India.

Dua R.P. (1992) Differential response of chickpea (Cicer arietinum) genotypes to salinity. *Journal of Agricultural Science* **119,** 367–371.

Dua R.P. (1997) Grafting technique in gram (*Cicer arietinum*) to ascertain control of root and shoot for salinity tolerance. *Indian Journal of Agricultural Sciences* **67,** 212–214.

Dua R.P. (1998) Salinity tolerance in chickpea (Cicer arientinum L.). *Indian Journal of Plant Physiology* **3,** 102–106.

Dua R.P. & Sharma P.C. (1995) Salinity tolerance of Kabuli and Desi chickpea genotypes. *International Chickpea and Pigeonpea Newsletter* **2,** 19–22.

Dua R.P. & Sharma S.K. (1997) Suitable genotypes of gram (Cicer arietinum) and mechanism of their tolerance to salinity. *Indian Journal of Agricultural Sciences* **67,** 440–443.

Elsheikh E.A.E. & Wood M. (1990a) Effect of salinity on growth, nodulation yield of chickpea (*Cicer arietinum* L.). *Journal of Experimental Botany* **41,** 1263–1269.

Elsheikh E.A.E. & Wood M. (1990b) Salt effects on survival and multiplication of chickpea and soybean rhizobia. *Soil Biology & Biochemistry* **22,** 343–347.

Epitalawage N., Eggenberg P. & Strasser R.J. (2003) Use of fast chlorophyll a fluorescence technique in detecting drought and salinity tolerant chickpea (*Cicer arietinum* L) varieties. *Archives Des Sciences* **56,** 79–93.

Esechie H.A., Al-Saidi A. & Al-Khanjari S. (2002) Effect of sodium chloride salinity on seedling emergence in chickpea. *Journal of Agronomy and Crop Science* **188,** 155–160.

Eyidogan F. & Oz M.T. (2007) Effect of salinity on antioxidant responses of chickpea seedlings. *Acta Physiologiae Plantarum* **29,** 485–493.

Fang X., Turner N.C., Yan G., Li F. & Siddique K.H.M. (2009) Flower numbers, pod production, pollen viability, and pistil function are reduced and flower and pod abortion increased in chickpea (*Cicer arietinum* L.) under terminal drought. *Journal of Experimental Botany* doi:10.1093/jxb/erp307.

FAO (2008) FAOSTAT. FAO.

Flowers T.J. & Yeo A.R. (1986) Ion relations of plants under drought and salinity. *Australian Journal of Plant Physiology* **13,** 75–91.

Foolad M.R. (2004) Recent advances in genetics of salt tolerance in tomato. *Plant Cell Tissue Organ Cult* **76,** 101–119.

Fricke W., Akhiyarova G., Veselov D. & Kudoyarova G. (2004) Rapid and tissue-specific changes in ABA and in growth rate in response to salinity in barley leaves. *Journal of Experimental Botany* **55,** 1115–1123.

Garcia A., Senadhira D., Flowers T.J. & Yeo A.R. (1995) The effects of selection for sodium transport and of selection for agronomic characteristics upon salt resistance in rice (*Oryza sativa* L). *Theoretical and Applied Genetics* **90,** 1106–1111.

Garg N. & Singla R. (2004) Growth, photosynthesis, nodule nitrogen and carbon fixation in the chickpea cultivars under salt stress. Brazilian. *Journal of Plant Physiology* **16,** 137–146.

Greenway H. & Osmond C.B. (1972) Salt responses of enzymes from species differing in salt tolerance. *Plant Physiology* **49,** 256–259.

Hernandez-Nistal J., Dopico B. & Labrador E. (2002) Cold and salt stress regulates the expression and activity of a chickpea cytosolic Cu/Zn superoxide dismutase. *Plant Science* **163,** 507–514.

Hunt S. & Layzell D. (1993) Gas exchange of legume nodules and the reduction of nitrogenase activity. *Annual Review of Plant Physiology and Molecular Biology* **44,** 483–511.

Johansen C., Saxena N.P., Chauhan Y.S., Subbrao G.V., Pundir R.P.S., Kumar Rao J.V.D.K. & Jana M.K. (1990) Genotypic variation in salinity response of chickpea and pigeonpea. In *Proceedings of the International Congress of Plant Physiology* (ed. S.K. Singh), pp. 977–983. Society of Plant Physiology and Biochemistry, New Delhi, India.

Karajeh F., Hamdy A., Bruggeman A., Touchan H. & Oweis T. (2003) *In vitro* salinity tolerance screening of some legumes and forages cultivars. I. In *Regional Action Programme (RAP): Water resources management and water saving in irrigated agriculture (WASIA PROJECT)* (ed. A. Hamdy), pp. 163–169. CIHEAM-IAMB, Bari, Italy.

Katerji N., van Hoorn J.W., Hamdy A., Mastrorilli M., Oweis T. & Malhotra R.S. (2001) Response to soil salinity of two chickpea varieties differing in drought tolerance. *Agricultural Water Management* **50,** 83–96.

Katerji N., van Hoorn J.W., Hamdy A., Mastrorilli M. & Oweis T. (2005) Salt tolerance analysis of chickpea, faba bean and durum wheat varieties – I. Chickpea and faba bean. *Agricultural Water Management* **72,** 177–194.

Kathira K., Nayagapara D.R., Vaddoria M.A. & Poshiya V.K. (1997) Screening of chickpea genotypes for salinity tolerance during germination and early seedling growth. *Gujurat Agricultural University Research Journal* **22,** 28–32.

Kaya M., Kaya G., Kaya M.D., Atak M., Saglam S., Khawar K.M. & Ciftci C.Y. (2008) Interaction between seed size and NaCl on germination and early seedling growth of some Turkish cultivars of chickpea (*Cicer arietinum* L.). *Journal of Zhejiang University-Science B* **9,** 371–377

Khalid M.N., Iqbal H.F., Tahir A. & Ahmad A.N. (2001) Germination potential of chickpeas (*Cicer arietinum*) under saline conditions. *Pakistan Journal of Biological Sciences* **4,** 395–396.

Kim S. & Copeland L. (1996) Enzymes of poly-β-hydroxybutyrate metabolism in soybean and chickpea bacteroids. *Applied and Environmental Microbiology* **62,** 4186–4190.

Kucuk C. & Kivanc M. (2008) Preliminary characterization of *Rhizobium* strains isolated from chickpea nodules. *African Journal of Biotechnology* **7,** 772–775.

Kukreja S., Nandwal A.S., Kumar N., Sharma S.K., Sharma S.K., Unvi V. & Sharma P.K. (2005) Plant water status, H2O2 scavenging enzymes, ethylene evolution and membrane integrity of *Cicer arietinum* roots as affected by salinity. *Biologia Plantarum* **49,** 305–308.

Kumar J., Gowda C.L.L., Saxena N.P., Sethi S.C., Singh U. & Sahrawat K.L. (1983) Effect of salinity on protein-content and seed size of chickpea (*Cicer arietinum* L). *Current Science* **52,** 82–83.

Kumar S. & Promila K. (1983) Effects of chloride and sulfate types of salinization and desalinization on nodulation and nitrogen-fixation in chickpea. *Indian Journal of Plant Physiology* **26,** 396–401.

Lauter D.J. & Munns D.N. (1986a) Salt resistance of chickpea genotypes in solutions salinized with NaCl or Na_2SO_4. *Plant & Soil* **95,** 271–279.

Lauter D.J. & Munns D.N. (1986b) Water-loss via the glandular trichomes of chickpea (*Cicer arietinum* L). *Journal of Experimental Botany* **37,** 640–649.

Lauter D.J. & Munns D.N. (1987) Salt sensitivity of chickpea during vegetative growth and at different humidities. *Australian Journal of Plant Physiology* **14,** 171–180.

Lauter D.J., Munns D.N. & Clarkin K.L. (1981) Salt response of chickpea as influenced by N supply. *Agronomy Journal* **73,** 961–966.

Lazzaro M.D. & Thomson W.W. (1989) Ultrastructure of organic-acid secreting trichomes of chickpea (Cicer arietinum). *Canadian Journal of Botany-Revue Canadienne De Botanique* **67,** 2669–2677.

Leport L., Turner N.C., Davies S.L. & Siddique K.H.M. (2006) Variation in pod production and abortion among chickpea

cultivars under terminal drought. *European Journal of Agronomy* **24,** 236–246.

Leport L., Turner N.C., French R.J., Tennant D., Thomson B.D. & Siddique K.H.M. (1998) Water relations, gas exchange and growth of cool-season grain legumes in a Mediterranean-type environment. *European Journal of Agronomy* **9,** 295–303.

L'Taief B., Sifi B., Zaman-Allah M., Drevon J.J. & Lachaal M. (2007) Effect of salinity on root-nodule conductance to the oxygen diffusion in the *Cicer arietinum Mesorhizobium ciceri* symbiosis. *Journal of Plant Physiology* **164,** 1028–1036.

Maliro M.F.A., McNeil D., Redden B., Kollmorgen J.F. & Pittock C. (2008) Sampling strategies and screening of chickpea (*Cicer arietinum* L.) germplasm for salt tolerance. *Genetic Resources and Crop Evolution* **55,** 53–63.

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. *Journal of Agronomy and Crop Science Zeitschrift Fur Acker und Pflanzenbau* **176,** 235–247.

Manchanda H.R. & Sharma S.K. (1989) Tolerance of chloride and sulphate salinity in chickpea (Cicer arietinum). *Journal of Agricultural Science* **113,** 407–410.

Manchanda H.R. & Sharma S.K. (1990) Influence of different chloride – sulfate ratios on yield of chickpea (*Cicer arietinum*) at comparable salinity levels. *Indian Journal of Agricultural Sciences* **60,** 553–555.

Mantri N.L., Ford R., Coram T.E. & Pang E.C.K. (2007) Transcriptional profiling of chickpea genes differentially regulated in response to high-salinity, cold and drought. *BMC Genomics* **8,** Article Number 303.

Mer R.K., Prajith P.K., Pandya D.H. & Pandey A.N. (2000) Effect of salts on germination of seeds and growth of young plants of Hordeum vulgare, *Triticum aestivum, Cicer arietinum* and Brassica juncea. Journal of Agronomy and Crop Science-Zeitschrift. *Fur Acker Und Pflanzenbau* **185,** 209–217.

Molina C., Rotter B., Horres R., *et al.* (2008) SuperSAGE: the drought stress-responsive transcriptome of chickpea roots. *BMC Genomics* **9,** 553.

Munns R. & Tester M. (2008) Mechanisms of salinity tolerance. *Annual Review of Plant Biology* **59,** 651–681.

Murumkar C.V. & Chavan P.D. (1986) Influence of salt stress on biochemical processes in chickpea, *Cicer arietinum* L. *Plant and Soil* **96,** 439–443.

Murumkar C.V. & Chavan P.D. (1993) Alterations in photosynthetic carbon metabolism of chickpea (*Cicer arietinum* L) due to imposed nacl salinity. *Agrochimica* **37,** 26–32.

Nandwal A.S., Kukreja S., Kumar N., Sharma P.K., Jain M., Mann A. & Singh S. (2007) Plant water, status, ethylene evolution, N-2-fixing efficiency, antioxidant activity and lipid peroxidation in *Cicer arietinum* L. nodules as affected by short-term salinization and desalinization. *Journal of Plant Physiology* **164,** 1161–1169.

Nene Y.L. & Reddy M.V. (1987) Chickpea diseases and their control. In *The Chickpea* (eds M.C. Saxna & K.B. Singh), pp. 233–270. CAB International, Wallingford, UK.

Nicolas C., DePrada J.M., Lorenzo O., Nicolas G. & Rodriguez D. (1998) Abscisic acid and stress regulate the expression of calmodulin in germinating chick-pea seeds. *Physiologia Plantarum* **104,** 379–384.

Pandey R. & Ganapathy P.S. (1985) The proline enigma :NaCl-tolerant and NaCl- sensitive callus lines of Cicer arietinum. *Plant Science* **40,** 13–17.

Rao D.L.N. & Sharma P.C. (1995a) Effectiveness of rhizobial strains for chickpea under salinity stress and recovery of nodulation on desalinization. *Indian Journal of Experimental Biology* **33,** 500–504.

Rao D.L.N., Giller K.E., Yeo A.R. & Flowers T.J. (2002) The effects of salinity and sodicity upon nodulation and nitrogen fixation in chickpea (Cicer arietinum). *Annals of Botany* **89,** 563–570.

Rao E.L.N. & Sharma P.C. (1995b) Alleviation of salinity stress in chickpea by *Rhizobium inoculation* or nitrate supply. *Biologia Plantarum* **37,** 405–410.

Reed W., Cardona S., Sithanantham S. & Lateef S.S. (1987) The chickpea insect pests and their control. In *The Chickpea* (eds M.C. Saxena & K.B. Singh), pp. 283–318. CAB International, Wallingford, UK.

Rengasamy P. (2002) Transient salinity and subsoil constraints to dryland farming in Australian sodic soils: an overview. *Australian Journal of Experimental Agriculture* **42,** 351–361.

Rengasamy P. (2006) World salinization with emphasis on Australia. *Journal of Experimental Botany* **57,** 1017–1023.

Rengasamy P., Chittleborough D. & Helyar K. (2003) Root-zone constraints and plant-based solutions for dryland salinity. *Plant and Soil* **257,** 249–260.

Richards R.A. (1983) Should selection for yield in saline regions be made on saline or non -saline soils. *Euphitica* **32,** 431–438.

Richter C., Heiligtag B., Nagieb M. & Mukhallalati U. (1999) The effects of salinity on growth and ion content of chickpea (*Cicer arietinum* L.), eggfruit (*Solanum melongena* L.) and red pepper (*Capsicum annuum* L.) varieties. *Tropenlandwirt* **100,** 3–16.

Rupela O. (1987) Nodulation and nitrogen fixation in chickpea. In *The Chickpea* (eds M. Saxena & K.B. Singh), pp. 191–206. CAB International, Wallingford, UK.

Sadiki M. & Rabih K. (2001) Selection of chickpea (Cicer arietinum) for yield and symbiotic nitrogen fixation ability under salt stress. *Agronomie* **21,** 659–666.

Sairam R., Tyagi A. & Chinnusamy V. (2006) Salinity tolerance: cellular mechanisms and gene regulation. In *Plant-Environment Interactions* (ed. B. Huang), pp. 121–175. CRC Press, Boca Raton, FL, USA.

Saxena A.K. & Rewari R.B. (1991) Influence of phosphate and zinc on growth, nodulation and mineral composition of chickpea (*Cicer arietinum* L.) under salt stress World. *Journal of Microbiology and Biotechnology* **7,** 202–205.

Saxena A.K. & Rewari R.B. (1992) Differential responses of chickpea (*Cicer arietinum* L) – *Rhizobium* combinations to saline soil-conditions. *Biology and Fertility of Soils* **13,** 31–34.

Saxena A.K. & Rewari R.B. (1993) Effect of potassium on the growth and nodulation of chick pea (Cicer arietinum L) under different levels of salinity. *Tropical Agriculture* **70,** 372–374.

Saxena N.P., Saxena M.C., Ruckenbauer P., Rana R.S., El-fouly M.M. & Shabana R. (1994) Screening techniques and sources of tolerance to salinity and mineral nutrient imbalances in cool season food legumes. *Euphytica* **73,** 85–93.

Sekhon B.S., Sandhu H.S., Dhillon K.S. & Singh R. (1987) Effect of salinity and nitrogen on nitrogenase and nitrate reductase activities in chickpea Indian Journal of. *Ecology* **14,** 289–291.

Serraj R., Krishnamurthy L. & Upadhyaya H.D. (2004) Screening chickpea mini-core germplasm for tolerance to soil salinity. *International Chickpea and Pigeonpea Newsletter* **11,** 29–32.

Shackel K.A. & Turner N.C. (2000) Seed coat turgor in chickpea is independent of changes in plant and pod water potential. *Journal of Experimental Botany* **51,** 895–900.

Sharma S.K. & Kumar S. (1990) Effect of salinization on growth and distribution of Na+ and Cl- in two genotypes of chickpea. *Indian Journal of Plant Physiology* **33,** 269–274.

Sharma S.K. & Kumar S. (1992) Effects of salinity on Na^+, K+, and Cl^- content in different organs of chickpea, and the basis of ion expression. *Biologia Plantarum* **34,** 311–317.

Sharma S.K., Singh D.K. & Dua R.P. (2004) Genetic divergence ib chickpea under saline conditions. *Indian Journal of Pulses Research* **17,** 80–81.

Sheldon A., Menzies N.W., So H.B. & Dalal R. (2004) The effect of salinity on plant available water. In *SuperSoil 2004*. CDROM, Available at: http://www.regional.org.au/au/asssi/supersoil2004/s6/poster/1523_sheldona.htm, University of Sydney.

Sheokand S., Dhandi S. & Swaraj K. (1995) Studies on nodule functioning and hydrogen peroxide scavenging enzymes under salt stress in chickpea nodules. *Plant Physiology and Biochemistry* **33,** 561–566.

Sheoran I.S. & Garg O.P. (1983) Effect of different types of salinity on gram (*Cicer arietinum* L.) during germination 1. Seedling growth and water relations. *Indian Journal of Pulses Research* **26,** 363–369.

Siddique K.H., Johansen C., Kumarrao J.V.D.K. & Ali M. (2005) *Legumes in sustainable cropping systems*. Paper presented at The Fourth International Food Legumes Research Conference, New Delhi – India.

Singh A.K., Singh R.A. & Sharma S.G. (2001) Salt stress induced changes in certain organic metabolites during seedling growth in chickpea. *Legume Research* **24,** 11–15.

Singh B., Singh B.K., Kumar J., Yadav S.S. & Usha K. (2005) Effects of salt stress on growth, nodulation, and nitrogen and carbon fixation of ten genetically diverse lines of chickpea (*Cicer arietinum* L.). *Australian Journal of Agricultural Research* **56,** 491–495.

Singh K.B., Malhotra R.S., Halila M.H., Knights E.J. & Verma M.M. (1994) Current Status and Future Strategy in Breeding Chickpea for Resistance to Biotic and Abiotic Stresses. *Euphytica* **73,** 137–149.

Sinhgla R. & Garg N. (2005) Influence of salinity on growth and yield attributes in chickpea cultivars. *Turkish Journal of Agriculture and Forestry* **29,** 231–235.

Sohrabi Y., Heidari G. & Esmailpoor B. (2008) Effect of salinity on growth and yield of Desi and Kabuli chickpea cultivars. *Pakistan Journal of Biological Sciences* **11,** 664–667.

Soltani A., Galeshi S., Zeinali E. & Latifi N. (2002) Germination, seed reserve utilization and seedling growth of chickpea as affected by salinity and seed size. *Seed Science and Technology* **30,** 51–60.

Soussi M., Ocana A. & Lluch C. (1998) Effects of salt stress on growth, photosynthesis and nitrogen fixation in chick-pea (Cicer arietinum L.). *Journal of Experimental Botany* **49,** 1329–1337.

Soussi M., Lluch C. & Ocana A. (1999) Comparative study of nitrogen fixation and carbon metabolism in two chick-pea (*Cicer arietinum* L.) cultivars under salt stress. *Journal of Experimental Botany* **50,** 1701–1708.

Soussi M., Khadri M., Lluch C. & Ocana A. (2001) Carbon metabolism and bacteroid respiration in nodules of chick-pea (*Cicer arietinum* L.) plants grown under saline conditions. *Plant Biosystems* **135,** 157–163.

Tanji K. (ed.) (1990) *Agricultural Salinity Assessment and Management*. American Society of Civil Engineers, New York.

Tejera N.A., Soussi M. & Lluch C. (2006) Physiological and nutritional indicators of tolerance to salinity in chickpea plants growing under symbiotic conditions. *Environmental and Experimental Botany* **58,** 17–24.

Vadez V., Krishnamurthy L., Serraj R., Gaur P.M., Upadhyaya H.D., Hoisington D.A., Varshney R.K., Turner N.C. & Siddique K.H.M. (2007) Large variation in salinity tolerance in chickpea is explained by differences in sensitivity at the reproductive stage. *Field Crops Research* **104,** 123–129.

Varshney R.K., Graner A. & Sorrells M.E. (2005) Genomics-assisted breeding for crop improvement. *Trends in Plant Science* **10,** 621–630.

Varshney R.K., Hoisington D.A., Upadhyaya H.D., *et al.* (2007) Molecular genetics and breeding of grain legume crops for the semi-arid tropics. In *Genomics Assisted Crop Improvement, Vol II: Genomics Applications in Crops* (eds R.K. Varshney & R. Tuberosa), pp. 207–242. Springer, Dordrecht, The Netherlands.

Varshney R.K., Close T.J., Singh N.K., Hoisington D.A. & Cook D.R. (2009a) Orphan legume crops enter the genomics era! *Current Opinions in Plant Biology* **12,** 202–210.

Varshney R.K., Hiremath P.J., Lekha P.T., *et al.* (2009b) A comprehensive resource of drought- and salinity- responsive ESTs for gene discovery and marker development in chickpea (*Cicer arietinum* L.). *BMC Genomics* **10,** 523 (Communicated).

Whish J.P.M., Castor P., Carberry P.S. & Peake A.S. (2007) On-farm assessment of constraints to chickpea (*Cicer arietinum*) production in marginal areas of northern. *Australia. Experimental Agriculture* **43,** 505–520.

Wilson J.R., Haydock K.P. & Robins M.F. (1970) The development in time of stress effects in two species of *Glycine* differing in sensitivity to salt. *Australian Journal of Biological Sciences* **23,** 537–551.

Wolfe O., Jeschke W.D. & Hartung W. (1990) Long distance transport of abscisic acid in NaCl-treated intact plants of Lupinus albus. *Journal of Experimental Botany* **41,** 593–600.

Yadav H.D., Yadav O.P., Dhankar O.P. & Oswal M.C. (1989) Effect of chloride salinity and boron on germination, growth and mineral composition of chickpea (*Cicer arietinum* L.). *Annals of Arid Zone* **28,** 63–68.

Yeo A.R., Yeo M.E., Flowers S.A. & Flowers T.J. (1990) Screening of rice (*Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance. *Theoretical and Applied Genetics* **79,** 377–384.

Zurayk R., Adlan M., Baalbaki R. & Saxena M.C. (1998) Interactive effects of salinity and biological nitrogen fixation on chickpea (*Cicer arietinum* L.) growth. Journal of Agronomy and Crop Science-Zeitschrift. *Fur Acker Und Pflanzenbau* **180,** 249–258.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. The percentage germination of genotypes of chickpea to salts. The columns of the table list the salts used, their concentration mM) or conductivity dS m^{-1}; 10 dS m^{-1} is about 100 mM), the genotype and the percentage germination after the noted time when measured in solutions on filter) paper or agar or in soil.

Table S2. Proline accumulation in chickpea when subjected to salinity.

Table S3. Identification of genetic variability and salinity resistance in genotypes of chickpea.

Table S4. The effect of different types and levels of salinity on the yield and/or reproductive structures in chickpea.

Table S5. Chickpea genotypes mentioned in the text and tables with synonyms if known.

Table S6. List of some candidate genes identified in response to salinity stress in chickpea.

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