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The Effects of Salinity and Sodicity upon Nodulation and Nitrogen Fixation in Chickpea (*Cicer arietinum*)

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Production of grain legumes is severely reduced in salt-affected soils because their ability to form and maintain nitrogen-fixing nodules is impaired by both salinity and sodicity (alkalinity). Genotypes of chickpea, *Cicer arietinum*, with high nodulation capacity under stress were identified by field screening in a sodic soil in India and subsequently evaluated quantitatively for nitrogen fixation in a glasshouse study in a saline but neutral soil in the UK. In the field, pH 8·9 was the critical upper limit for most genotypes studied but genotypes with high nodulation outperformed all others at pH 9·0–9·2. The threshold limit of soil salinity for shoot growth was at ECe 3 dS m^{-1}, except for the high-nodulation selection for which it was ECe 6. Nodulation was reduced in all genotypes at salinities above 3 dS m^{-1} but to a lesser extent in the high-nodulation selection, which proved inherently superior under both non-saline and stress conditions. Nitrogen fixation was also much more tolerant of salinity in this selection than in the other genotypes studied. The results show that chickpea genotypes tolerant of salt-affected soil have better nodulation and support higher rates of symbiotic nitrogen fixation than sensitive genotypes.

Key words: *Cicer arietinum* L., chickpea, Fabaceae, legume, salinity, sodicity, rhizobia, nitrogen fixation, nodulation.

INTRODUCTION

Salinity in the arid and semi-arid regions of the world is a serious threat to agriculture. Production of grain legumes is particularly vulnerable because of their low tolerance to salinity combined with the high sensitivity of the initiation of symbiotic nitrogen fixation to stress: infection of root hairs by rhizobia and subsequent nodule development are particularly sensitive to salinity. Salinity does not affect colonization of roots by rhizobia (Singleton and Bohlool, 1984) but does retard initiation or growth of new nodules, reduce the efficiency of fully formed nodules which had developed earlier under non-saline conditions (Bernstein and Ogata, 1966) and decrease the proportion of those nodules that *are* initiated in saline conditions that are able to differentiate fully into active N_2-fixing nodules (Yousef and Sprent, 1983).

Cicer arietinum is one of the most important grain legumes grown in semi-arid regions. Cultivars grown in India are either native (‘desi’) types or Mediterranean (‘kabuli’) types. The ‘kabuli’ types have been found to be more tolerant of salinity than the ‘desi’ types (Dua and Sharma, 1995). However, the nodulation pattern of the two groups in saline soils has not previously been examined and the role of the bacterial symbiont in overall yield in salt-affected soil remains controversial. Over 25 years ago, Bhardwaj (1975) showed that the salinity and alkalinity tolerance limits of *Rhizobium* and *Bradyrhizobium* spp. are much higher than those of the legume host, which suggested that it is predominantly the tolerance of the host plant that dictates the possibility of a successful symbiosis. A salt-sensitive soybean cultivar ‘William’ performed poorly whether a salt-tolerant or a salt-sensitive *Bradyrhizobium* strain was used for inoculation (Velagaleti and Marsh, 1989). A salt-tolerant soybean host cultivar ‘Manchu’ sustained nodulation irrespective of the salt tolerance of the strain of bacterium; no significant differences in shoot or root dry weight, nodule number or nodule weight were found whether a salt-sensitive *Bradyrhizobium* strain or a salt-tolerant *Rhizobium* strain was used for inoculation (Elsheikh and Wood, 1995). However, Saxena and Rewari (1992), using the classical ‘host × genotype interaction’ strategy, concluded that *C. arietinum* yields can be improved substantially in saline soils by selecting for both a salt-tolerant host and an appropriate *Rhizobium* strain. It was subsequently shown in a study of a single host in combination with eight individual strains of rhizobia that the strains most effective in non-saline conditions were also the most effective in salt-affected soil (Rao and Sharma, 1995). This suggests that the key feature in sustaining nitrogen fixation under salt stress is the nodulation ability of the host genotype.

Here we report the screening of *C. arietinum* genotypes for nodulation and grain-yielding ability in a sodic field

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over two growing seasons in order to identify genotypes with superior nodulation ability under stress. Selected genotypes, covering a range of tolerance, were then evaluated for the effect of salinity upon the relative importance of different (soil and atmospheric) sources of nitrogen to the plant using the technique of ^{15}N isotope dilution.

MATERIALS AND METHODS

Plant material

Genotypes of *Cicer arietinum* L. used for field evaluation were from the germplasm collection held at the Central Soil Salinity Research Institute (CSSRI, Indian Council of Agricultural Research), Haryana, India: seed samples were taken from varietal trial plots. The genotypes evaluated were: CSG 8890, CSG 8893, CSG 8943, CSG 8962, CSG 9001, CSG 9002, CSG 9017, CSG 9113, CSG 9372, CSG 88101, C 235, PBG 1 and BG 256. Detailed data are presented on five genotypes selected as representing known salt-tolerant (CSG 8962) and salt-sensitive (CSG 8890) genotypes, as well as a high-nodulation selection (CSG 9372) and two commonly grown cultivars (BG 256 and C 235) as checks.

For measurements of nitrogen fixation, the genotypes used were: (1) ICC 4918, a non-N-fixing (nod$^-$) control (Rupela, 1992) obtained from ICRISAT, Hyderabad, India; (2) CSG 8890, a salt-sensitive genotype (Dua and Sharma, 1995); (3) BG 256, a high yielding and commonly cultivated variety (check); (4) CSG 9372, a superior nodulating selection; and (5) CSG 8927, a salt-tolerant genotype (Dua and Sharma, 1995). The genotypes (1) and (2) were of the small-seeded 'desi' type, (3) was medium-seeded 'desi', (4) was bold-seeded 'desi' and (5) was of the Mediterranean or 'kabuli' type.

Screening of C. arietinum *genotypes for nodulation in a sodic field*

Screening was conducted for two growing seasons (1993, 1994) to assess the genotypic differences in nodulation, ion uptake, nitrogen uptake and yield. The experiment was performed in a sodic (alkali) area in an experimental farm (CSSRI) at Kaithal, Haryana, India. The soil was an alluvial sandy loam, typic natrustalf. The pH (1 : 2, soil : water) in the 0–15 cm soil layer was 8·8 and in the 15–30 cm soil layer was 9·1. Salinity (EC_2) was 0·55 and 0·44 dS m^{-1} in the upper and lower soil layers, respectively. After application of a pre-sowing irrigation with water drawn from a tube-well, the land was ploughed, levelled and bunded to make three beds each 40 m × 2 m, separated from each other by a 1-m wide path.

In October of each year of the experiment, genotypes were sown by hand in lines in a 3 m × 1 m plot with 30 cm between rows and 10 cm between plants within rows. There were thus 124 plants in each plot. Of the four rows, the two outer rows were sampled to assess nodulation at 40 and 90 d after sowing by carefully uprooting four plants chosen at random and gently washing the roots with tap water to remove the nodules, which were then counted and weighed. The two central rows were harvested for biomass and grain yield per plant in April of the following year. The nitrogen concentration of the shoots was measured and multiplied by the dry mass to give nitrogen content (the total quantity of nitrogen in the plant). Sodium and potassium concentrations were also measured.

Quantification of nitrogen fixation by isotope dilution

Nitrogen fixation was quantified in an experiment using plants grown in pots in a saline but neutral pH soil at Wye College, University of London, UK. The soil used was sampled from the Al horizon (8–20 cm layer) of a brown earth collected from a freely draining, unfertilized site located within Forestry Commission plots at Challock, Kent, UK. The soil was collected after a period of rainfall to ensure that the concentrations of nitrate-nitrogen that would have formed during a period without rain had been reduced by leaching. Soil was spread out in a glasshouse for 24 h to reduce its moisture content and facilitate handling, but it was not allowed to dry out completely. The soil was hand-crumbled, stones and large roots were removed manually, and the soil was sieved through a 4-mm mesh to remove fine roots. The soil was acidic (pH = 4·3, 1 : 2·5 soil : water) and exchangeable acidity was 11·25 $cmol_c$ kg^{-1} soil. The soil was limed by applying 5·5 g $CaCO_3$ kg^{-1} soil. After neutralization, the soil was analysed for texture (sandy clay loam), pH 7·0, 1 : 2, ECe (1·0 dS m^{-1}), cation exchange capacity [20·4 $cmol_c$ kg^{-1} (centimoles per kg, the sub-script 'c' refers to cations; numerically equal to meq per 100 g)], organic carbon (1·7 %), total nitrogen (0·12 %), available phosphorus (27·6 mg kg^{-1}) and available potassium (80·0 mg kg^{-1}). Three parts of soil were mixed with one part of silver sand, followed by incorporation of a nutrient mixture (modified from Arnon and Hoagland, without nitrogen; Subbarao *et al*., 1990) and ^{15}N-labelled ammonium sulfate (10·5 atom % excess) at 5 ppm. This soil mixture (ECe 1·0 dS m^{-1}) was salinized with a mixed salt solution consisting of NaCl, Na_2SO_4, $CaCl_2{\cdot}6H_2O$ and $MgCl_2{\cdot}6H_2O$, to varying electrical conductivities while maintaining a Cl : SO_4 ratio of 3 : 1, a Ca : Mg ratio of 1 : 1·5 and a sodium adsorption ratio of 10 (all values representative of a saline-sodic soil) across all salinity treatments. The salinity treatments created were ECe 3·2, 6·2 and 8·1 dS m^{-1}. Soil (750 g, corresponding to 650 g dry soil at 12·5 % moisture content) was placed in 1 kg capacity plastic pots. Seeds were surface sterilized in 95 % ethanol for 30 s and 3 % sodium hypochlorite for 3 min, followed by repeated washing in sterile distilled water, soaked overnight and sown in pots on 10 Oct. 1995. Four seeds were sown per pot, with five replicates of each salinity treatment. After emergence, 10-d-old broth cultures of *C. arietinum* rhizobial strains IC 21, IC 26, IC 53 from the ICRISAT collection (obtained from IARI, New Delhi, India) and Ca-181 and Ca-121 (obtained from the Department of Microbiology, Haryana Agricultural University, Hisar, India) were used for inoculation. These were the strains found to be most effective in the earlier study of Rao and Sharma (1995) and were used together to ensure the potential for nodulation in a soil (from

the UK), which may or may not have contained appropriate rhizobia for *C. arietinum*. Each strain was diluted 1·5-times with distilled water and 10 ml of the suspension was pipetted onto the soil surface of each pot followed by irrigation with 10 ml distilled water.

The pots (120 in all) were arranged in five replicate blocks across the glasshouse bench. In each block, genotypes were randomized in the 'vertical' direction and then salinity levels were randomized in the 'horizontal' direction to minimize any effects of spatial variation within the glasshouse. A 12 h light (natural daylight extended by artificial illumination) and 12 h dark cycle, and a day/night temperature of 22/18 °C was maintained throughout the experimental period. The pots were watered on alternate days by gently spraying with deionized water. Differences in moisture content in each pot due to differential evapotranspiration losses in different genotypes and at different salinity levels were compensated for once a week by weighing each pot and adding the requisite amount of water to bring the soil moisture content to 70 % field capacity. After 10 d, plants were thinned to two plants per pot. The harvested plant material was chopped up and returned to the soil to allow it to decompose so that no labelled nitrogen was lost from the system. Biological plant protection measures were taken to prevent insect attack on plants. After 60 d of growth, shoots were harvested, dried in an oven at 60 °C and weighed. The soil in each pot was washed over a nest of sieves to recover roots, and the nodules were detached and counted. The roots and nodules were dried and weighed. The shoot material was finely ground in a ball mill and a sample analysed for total N and ^{15}N enrichment using a Roboprep C/N analyser coupled to a Micromass 622 mass spectrometer.

The %N from N_2 fixation from the glasshouse data was calculated by the isotope dilution method:

$$\%\text{N from } N_2 \text{ fixation} = [1 - (R_{\text{nodulated legume}}/R_{\text{reference plant}}) \times 100]$$

where R = atom % ^{15}N excess, and the total amount of N from fixation was calculated as:

$$\text{total N fixed} = (\%\text{N from } N_2 \text{ fixation} \times N_p)/100$$

where N_p is total plant nitrogen.

Analysis of ions

Sodium, potassium, calcium and magnesium were estimated in samples from the shoot by atomic absorption spectrometry (Unicam 919) and chloride, sulfate, phosphate and nitrate by ion exchange chromatography (Dionex DX 100, Camberley, UK). Anions were separated using an 'IONPAC' AS14 analytical column with a corresponding AG14 mm guard column. Eluent anions were suppressed with a self-regenerating suppressor (ASRS-ULTRA-4 mm).

Statistical analysis

Results of the field and pot experiments were analysed by ANOVA using a randomized block design model of the MINITAB statistical package.

RESULTS AND DISCUSSION

Nodulation and growth in the field

Spatial variation in soil properties over the field (typical of salt-affected soils) meant that there were slight differences in pH between the three replicate blocks (blocks 1, 2 and 3 having pH values of 9·0, 9·2 and 8·9, respectively). Plants at pH 8·9 showed the least effects of stress, and their nodules were healthy and pink. At pH 9·0 and 9·2 the nodules became black in colour and were damaged. A pH of 8·9 was thus the critical limit for nodulation in *C. arietinum*. Above pH 8·9, nodule biomass reduced drastically (data not shown); even at pH 8·9 the sensitive genotype CSG 8890 had the lowest nodule biomass (70 mg per plant at 90 d after sowing) compared with the other genotypes [whose biomass ranged from 101–330 (average 242 ± 85) mg per plant). CSG 9372 (high nodulating) and CSG 8962 (salt tolerant) had the greatest nodule biomass at 90 d (Table 1). At pH 9·0 and 9·2, CSG 9372 showed the best performance in terms of nodulation (data not shown). There were no significant differences among the genotypes in Na, K and N concentrations in shoots after 90 d (Table 1), although there were large differences in the quantity of N in the shoots and in shoot biomass.

At harvest, the total above-ground biomass (straw plus grain), which indicates the total growth response to the treatments without considering the additional variable of partitioning, clearly shows that BG 256 (21·2 g per plant) and CSG 9372 (21·3 g per plant) were in a (statistically significant; LSD P = 0·05, 6·9) separate class above CSG 8890 (10·3 g per plant) and C 235 (10·1 g per plant). The differences in grain yield among genotypes (whose nodulation was sensitive or tolerant to salinity) were not significant. This arose because of the spatial variability in the three replication blocks viz., pH 8·9, 9·0 and 9·2, and because a pH of 8·9 was not limiting to nodulation and so masked real differences. When grain yield data of two replication blocks of limiting pH (pH 9·0 and 9·2) were analysed, the sensitive genotypes could be identified and fell into three clusters with the following relative ranking (Duncan's multiple range test): CSG 9372 = BG 256 = CSG 8962 > C 235 > CSG 8890 (error mean square 5·1, LSD 4·2 g per plant). This further confirmed the previous observation of a critical pH limit for nodulation and its role in influencing grain yield of legumes.

There was a strong positive relationship between grain yield and N content (mg per plant) over all genotypes (R^2 = 92 %, P = 0·011) and between grain yield and shoot biomass (R^2 = 89 %, P = 0·017; data in Table 1). There was no significant regression at 90 d of nodule weight on nodule number (R^2 = 24 %, P = 0·23). At 90 d, N content was strongly related to the number of nodules per plant (R^2 = 82 %, P = 0·034) but not significantly related to nodule biomass per plant (R^2 = 16 %, P = 0·48). N uptake was higher in the higher yielding genotypes. Further experiments were conducted to determine whether this increased N uptake was due to higher nitrogen fixation or simply due to greater uptake of N from the soil under the salt-stress conditions.

TABLE 1. *Characteristics of five selected genotypes of* Cicer arietinum *grown in a sodic field (pooled across soil pH values) at Kaithal in India*

Genotype	Nodules (no. per plant)	Nodule dry mass (mg per plant)	Shoot dry mass (g per plant)	Shoot N (% dry mass)	Shoot Na (% dry mass)	Shoot K (% dry mass)	Shoot N content (mg per plant)	Grain yield (g per plant)	Straw yield (g per plant)
Plants harvested 40 days after sowing									
CSG 8890 (salt-sensitive)	1·7	6·8	0·22	3·2	0·30	2·1	7·1		
BG 256 (check)	2·8	10·9	0·32	2·9	0·26	1·9	9·1		
C 235 (check)	2·7	10·8	0·21	3·3	0·29	2·2	7·0		
CSG 9372 (high-nodulation)	4·5	16·7	0·36	3·5	0·32	1·9	12·4		
CSG 8962 (salt-tolerant)	2·9	10·0	0·21	3·2	0·32	2·3	6·8		
LSD $P = 0{\cdot}05$	N.S.	N.S.	0·14	0·6	N.S.	0·2	4·2		
Plants harvested 90 (153 for grain and straw) d after sowing									
CSG 8890 (salt-sensitive)	1·3	71	1·9	2·5	0·30	1·7	47·4	5·2	5·0
BG 256 (check)	4·7	128	3·2	3·1	0·38	1·7	99·9	9·9	11·3
C 235 (check)	2·4	130	1·7	3·4	0·29	1·4	59·8	5·4	4·7
CSG 9372 (high-nodulation)	7·1	209	3·8	2·5	0·32	1·6	94·3	8·7	12·6
CSG 8962 (salt-tolerant)	2·7	200	2·0	2·8	0·33	1·5	59·1	6·9	8·0
LSD $P = 0{\cdot}05$	3·9	N.S.	1·6	0·9	N.S.	N.S.	N.S.	N.S.	2·2

Grain and straw yield are at harvest, 153 d after sowing.
N.S., not significant.

TABLE 2. *Shoot and root dry mass of five selected genotypes and nodule number and dry mass of nodules of four (the non-nodulating ICC 4918 does not form part of the analysis) selected genotypes of* C. arietinum *grown in an artificial saline soil at Wye College, UK*

Genotype	Shoot dry mass (g per pot)				LSD $P = 0{\cdot}05$	Root dry mass (g per pot)				LSD $P = 0{\cdot}05$
ECe (dS m^{-1})	1·0	3·2	6·2	8·1	0·10 (among ECe)	1·0	3·2	6·2	8·1	0·05 (among ECe)
ICC 4918 (non-nodulating)	0·57	0·61	0·50	0·14		0·26	0·21	0·15	0·03	
CSG 8890 (salt-sensitive)	0·76	0·70	0·50	0·32		0·50	0·37	0·19	0·11	
BG 256 (check)	1·11	0·90	0·65	0·47		0·46	0·41	0·24	0·13	
CSG 9372 (high-nodulation)	1·03	1·22	1·14	0·60		0·38	0·46	0·38	0·22	
CSG 8927 (salt-tolerant)	0·93	0·91	0·60	0·54		0·43	0·47	0·31	0·20	
LSD $P = 0{\cdot}05$	0·12 (among genotypes)					0·06 (among genotypes)				
	Number of nodules per pot					Dry mass of nodules (mg per pot)				
CSG 8890 (salt-sensitive)	23·0	0·0	5·6	7·6	6·5 (among ECe)	8·9	1·7	0·7	0·5	2·7 (among ECe)
BG 256 (check)	22·2	11·8	13·8	7·6		12·8	5·2	3·3	1·2	
CSG 9372 (high-nodulation)	63·2	20·8	21·8	13·0		15·8	5·1	2·8	1·1	
CSG 8927 (salt-tolerant)	25·2	15·6	7·2	2·6		5·9	4·4	1·4	0·3	
LSD $P = 0{\cdot}05$	6·5 (among genotypes)					2·7 (among genotypes)				

Plants were harvested 60 d after sowing.

TABLE 3. *Ion concentrations (means of five selected genotypes of* C. arietinum*) grown on an artificial saline soil at Wye College, UK*

ECe (dS m^{-1})	Na (μmol g^{-1})	K (μmol g^{-1})	Ca (μmol g^{-1})	Mg (μmol g^{-1})	Cl (μmol g^{-1})	SO_4 (μmol g^{-1})	P_i (μmol g^{-1})	NO_3 (μmol g^{-1})
1·0	193	176	577	86·4	86·4	40·2	21·5	1·3
3·2	167	202	572	76·9	697	51·5	20·1	0·5
6·2	209	178	453	67·8	996	46·0	22·2	0·8
8·1	289	294	465	76·9	1158	47·7	32·5	2·4
LSD $P = 0{\cdot}05$	55	36	80	N.S.	69·1	N.S.	3·2	0·8

Plants were harvested 60 d after sowing. Data are on a shoot dry mass basis.
P_i, Phosphate.

Growth in pots

Four genotypes were selected (see Materials and Methods) for determination of nitrogen fixation in a pot experiment, together with the non-nodulating genotype ICC 4918 as a control. The threshold salinity level (highest ECe at which growth is not significantly depressed with respect to a non-saline control) was ECe 6·2 dSm^{-1} in the high-nodulating selection and ECe 3·2 in the four other genotypes. Working with sensitive genotypes of *C. arietinum*, Lauter *et al*. (1981) showed that growth was depressed even at 20 mM NaCl (EC approx. 2 dS m^{-1}). Results on root growth (Table 2), and therefore total dry mass production, showed a similar trend to shoot dry mass. At ECe 6·2 dS m^{-1}, total plant dry mass (shoot + root) of CSG 9372 was greater than that of all other genotypes and at ECe 8·1 dS m^{-1} the total dry mass of the high-nodulating and salt-tolerant genotypes were not significantly different (Table 2). Averaged across all salinity levels, BG 256 (1·09 g per plant), CSG 9372 (1·36 g per plant) and CSG 8927 (1·10 g per plant) out-performed CSG 8890 (0·86 g per plant; LSD $P = 0{\cdot}05$, 0·17) in total growth (Table 2).

Ion concentrations in the chickpea shoots

There were no significant differences in the concentrations of sulfate, phosphate, nitrate, sodium, calcium and magnesium (expressed on a dry weight basis) in the shoots of the various genotypes growing in any one treatment. There were small differences among genotypes only in potassium and chloride concentrations (differences between genotypes differing in sensitivity to salt are probably due to differences in the ability of the roots to retain ions and of the shoots to maintain their water contents; R. P. Dua, pers. comm.). The major differences in ion concentration were found at different salinities; hence, mean data across all genotypes at different salinity levels are presented in Table 3. As salinity increased, the concentration of sodium, chloride, nitrate and phosphate in the shoot increased, and the concentration of calcium decreased; these differences were generally significant only at the higher salinities (Table 3). Dua and Sharma (1997) also reported increased chloride and sodium but stable potassium concentrations in plants as salinity increased. It is difficult to separate these increases in concentration from the 'concentrative'

TABLE 4. *Shoot nitrogen concentration, shoot nitrogen content (quantity of N), % atom excess of ^{15}N in shoots, Ndfa and N_2-fixation in five selected genotypes of* C. arietinum *grown in an artificial saline soil at Wye College, UK*

Genotype					LSD P = 0·05
	Shoot nitrogen concentration (%)				
ECe (dS m^{-1})	1·0	3·2	6·2	8·1	0·26 (among ECe)
ICC 4918 (non-nodulating)	5·28	4·31	4·52	4·70	
CSG 8890 (salt-sensitive)	5·11	4·89	5·10	5·15	
BG 256 (check)	4·09	4·67	4·76	4·78	
CSG 9372 (high-nodulation)	4·92	4·55	4·80	5·29	
CSG 8927 (salt-tolerant)	5·54	5·01	5·42	5·50	
LSD P = 0·05	0·29 (among genotypes)				
	Shoot nitrogen content (mg per pot)				
ICC 4918 (non-nodulating)	30·2	26·4	22·7	6·3	4·3 (among ECe)
CSG 8890 (salt-sensitive)	38·5	33·9	25·5	16·2	
BG 256 (check)	43·8	40·9	30·3	22·2	
CSG 9372 (high-nodulation)	48·5	55·4	54·5	31·2	
CSG 8927 (salt-tolerant)	51·1	45·7	32·2	29·5	
LSD P = 0·05	4·8 (among genotypes)				
	% atom excess				
ICC 4918 (non-nodulating)	0·311	0·333	0·321	0·232	0·013 (among Ece)
CSG 8890 (salt-sensitive)	0·257	0·271	0·267	0·232	
BG 256 (check)	0·244	0·273	0·295	0·242	
CSG 9372 (high-nodulation)	0·222	0·226	0·231	0·243	
CSG 8927 (salt-tolerant)	0·220	0·246	0·249	0·216	
LSD P = 0·05	0·014 (among genotypes)				
	Nitrogen derived from fixation (%)				
CSG 8890 (salt-sensitive)	17·5	18·6	17·1	0·2	4·8 (among ECe)
BG 256 (check)	21·6	18·2	8·3	0·0	
CSG 9372 (high-nodulation)	28·6	32·1	28·2	0·0	
CSG 8927 (salt-tolerant)	29·2	26·1	22·5	7·1	
LSD P = 0·05	4·8 (among genotypes)				
	Nitrogen fixation (mg per pot)				
CSG 8890 (salt-sensitive)	6·7	6·3	4·4	0·0	2·3 (among ECe)
BG 256 (check)	9·5	7·4	2·5	0·0	
CSG 9372 (high-nodulation)	13·9	17·8	15·4	0·0	
CSG 8927 (salt-tolerant)	14·9	11·9	7·2	2·1	
LSD P = 0·05	2·3 (among genotypes)				

effect of biomass reduction with increasing salinity. The most striking differences were with respect to chloride, the concentration of which increased dramatically with increasing salinity and could not be accounted for by reduced biomass (Tables 2 and 3). Chloride changed from being a little under half the concentration of sodium in plants grown in the absence of sodium chloride to an average of 4·3-times that of sodium in salinized plants. Mamo *et al*. (1996) reported a similar (five-fold) difference for chickpea growing under salinized conditions. Although there was, in our data, a large disparity between chloride and sodium, the measured anions and cations were not dramatically out of balance due to the high concentrations of calcium in the tissues. If any consistent relationship between yield and ion concentration exists between genotypes, then chloride would be the most promising candidate ion with which to screen for salinity tolerance in *C. arietinum*.

Effect of salinity upon nodulation and nitrogen uptake and fixation

With increasing salinity, there was a sharp decrease in nodule number and nodule biomass in all genotypes, even at ECe 3·2 dS m^{-1}, relative to the 1·0 dS m^{-1} control (Table 2). In contrast to the field experiment, nodule number and nodule mass were significantly related: removing the 1·0 dS m^{-1} controls meant the regression had an R^2 of 49 % (P = 0·007). The threshold for these parameters was therefore less than the threshold value for shoot and root growth, and confirmed the greater sensitivity of nodulation, compared with vegetative growth, to salinity. In *Vicia*, Yousef and

Sprent (1983) showed that total as well as specific nitrogenase activity of root nodules decreased with stress, the number and dry mass of nodules decreased, plant dry mass decreased, and the proportion of nodules that did form which differentiated into active nitrogen-fixing nodules also decreased due to salt stress. Velagaleti and Marsh (1989) concluded that nodule mass is significantly reduced under stress due to inadequate photosynthate supply to the roots caused by decreased plant dry mass production.

CSG 9372 was an inherently superior nodulating genotype. Under non-saline conditions, it had nearly three times the number of nodules of the other nodule-forming genotypes (Table 2). The number of nodules remained higher than all other genotypes at all salinities. However, the dry mass of root nodules did not differ significantly from the check (BG 256) nor the salt-tolerant CSG 8927 at 3·2 dS m^{-1} and 6·2 dS m^{-1}: at the highest salinity the nodule dry mass for all genotypes was very small.

Although nitrogen content (quantity) decreased with increasing salinity (significantly so in all genotypes), there was no consistent reduction in shoot nitrogen concentration (Table 4). This implies either that reduction in shoot growth had a concentrative effect, which compensated for nitrogen uptake and fixation, or else that shoot growth was regulated by N concentration. At the highest salinity the nitrogen content of the nod$^-$ ICC 4918 was more seriously affected (reduced by 80 % with respect to the control) than the symbiotic genotypes (maintaining on average 50 % of the N content of the control), suggesting that dependence upon a mineral source of nitrogen may have been a disadvantage in conditions of the greatest competition for ion uptake. Overall, the results suggest that genotypes with the greatest capacity for nodulation perform best under both unstressed and stressed conditions.

Effect of salinity on nitrogen fixation

The greater nitrogen content of CSG 9372 (significant at all salinity treatments and with respect to all other genotypes except CSG 8927 at 8·1 dS m^{-1}) could be due to better nitrogen fixation and/or better nitrogen uptake from the soil under salt stress. The lower enrichment with labelled nitrogen in CSG 9372 at ECe 6·2 dS m^{-1} (0·23 atom % excess, Table 4) compared with the check (0·29) and CSG 8927 (0·25), and hence higher dilution of soil-derived nitrogen, indicated that CSG 9372 had a superior N-fixing ability. At ECe 6·2 dS m^{-1}, CSG 9372 derived 28·2 % of its nitrogen from fixation compared with 8·3 % in the check and 22·4 % in the salt-tolerant CSG 8927 (Table 4). Nitrogen fixation was significantly lower in the check (BG 256) and salt-sensitive selection than in the salt-tolerant and high-nodulation selections under control (1·0 dS m^{-1}) conditions. The high-nodulation selection sustained no significant loss in N_2-fixation at ECe 6·2 dS m^{-1}, whereas all other genotypes did. The highest salinity level of 8·1dS m^{-1} was so inhibitive of the symbiotic process that all the genotypes exhibited negligible N_2-fixation in this treatment.

CONCLUSIONS

Overall, the results suggest that genotypes with the greatest capacity for nodulation perform best under both unstressed and stressed conditions in terms of both N fixation and grain yield. In the field at least, the number of nodules appeared more important than the dry mass of nodules. The results suggest that although host × symbiont interactions may occur in saline soils, this has little to do with the 'salt tolerance' of the bacterium. This means, in terms of plant breeding, that selection for improved nitrogen fixation should proceed by first screening legume germplasm to identify genotypes with the best available nodulation and grain yield characteristics under salt stress. This would then be followed by matching these promising genotypes to strains of rhizobia known to be most effective in stressed or non-stressed conditions in situations where these strains did not exist in sufficient numbers naturally in the soil. An ECe of 6·2 dS m^{-1} proved discriminatory for screening purposes.

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