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Alleviation of salinity stress in chickpea by *Rhizobium* inoculation or nitrate supply

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Abstract

Influence of inoculation with efficient rhizobia or nitrate fertilization in alleviating salinity (NaCl, $CaCl_2$ and Na_2SO_4) stress was investigated in sand culture experiments. Shoot dry mass declined beyond salinity level corresponding to electrical conductivity (EC) 5.6 dS m^{-1} in control or in inoculated plants and after EC 7.4 dS m^{-1} in nitrate fed ones. Root growth was more sensitive and decreased at EC 3.3 dS m^{-1}. Nitrate reductase activity in leaves reduced at EC 3.3 dS m^{-1} but in inoculated and nitrate fed plants it reduced at EC 5.6 dS m^{-1}. Na^+ accumulation increased at EC 5.6 and 7.4 dS m^{-1} in roots and shoots, respectively. In inoculated and nitrate fed plants Na^+ content in roots increased at EC 7.4 dS m^{-1}. Content of Ca^{2+} increased slightly only in shoots and content of K^+ was unaffected. Besides inoculation, application of small doses of nitrogen should prove beneficial for legume cultivation in saline soils.

Key words: *Cicer arietinum*, ion accumulation, nitrate reductase activity

Introduction

It is generally known that chickpea (*Cicer arietinum* L.), the most important pulse crop in North India, is sensitive to salinity. Growth of chickpea (Lauter *et al.* 1981) and soybean (Bernstein and Ogata 1966, Wilson 1970) was found even more affected by salinity when grown symbiotically as nodulation and nitrogen fixation was very sensitive to salinity (Wilson 1970, Bhardwaj 1975). However, in alfalfa growth inhibition was similar for nitrate fed and N_2-fixing plants (Bernstein and Ogata 1966). The aim of present study was to determine whether salinity stress in chickpea could be alleviated by *Rhizobium* inoculation and/or nitrate supply.

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Abbreviations: DAS - days after seeding; EC - electrical conductivity; NR - nitrate reductase

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Materials and methods

Five effective *Rhizobium* strains (IC 59, 76, 2002, 2009 and 2018) used in this study for inoculating chickpea were obtained from ICRISAT, Hyderabad, India. In laboratory studies they showed high *in vitro* tolerance to salinity (Rao and Sharma 1995). Since their individual symbiotic behaviour to salinity was not known, a mixed inoculum was used to ensure nodulation by one or more strains. Seeds of *Cicer arietinum* cv. Pusa C-256, (a cultivar sensitive to salinity - Saxena and Rewari 1992) were surface sterilized with 0.2 % $HgCl_2$ for 5 min, washed in sterile water and sown in pots filled with river sand. Salinization was done at 16 d after sowing (DAS) to maintain 4 levels (electrical conductivity 3.3, 5.6, 7.4 and 9.2 dS m^{-1}). For each salinity level, 3 nitrogen sub-treatments were imposed: *a*) minus N and uninoculated, *b*) minus N and inoculated with *Rhizobium* spp., and *c*) N fed, uninoculated. The experiment was conducted in randomized complete block design with 3 replications for each treatment.

A modified Arnon and Hoagland's N-free nutrient solution (1/4 strength, Subba Rao *et al.* 1990) with NaCl, $CaCl_2$ and Na_2SO_4 (Na^+:Ca^{2+} ratio 3:1 and Cl^-:SO_4^{2-} ratio 2.5:1) was used for various salinity treatments. The electrical conductivity of the nutrient solution without added salt was 0.8 dS m^{-1} (control). A mixed YEM broth culture of the 5 *Rhizobium* strains containing 10^8 cells cm^{-3} was inoculated 17 DAS (1 cm^3 per seedling). It was repeated at 20 and 23 DAS to ensure adequate population of rhizobia in the rhizosphere. The N fertilization (25 mg dm^{-3} N as NH_4NO_3) was given from 35 DAS. At the end of every 4 d evapo-transpirational losses were made up by adding deionized water. At 70 and 105 DAS the 5th fully expanded leaf from the top was taken to determine the *in vivo* nitrate reductase activity (Jaworski 1971). The chopped leaf tissue was suspended in reaction mixture comprising 5 % propanol and 0.02 M KNO_3 in 0.1 M phosphate buffer, pH 7.5. After dark incubation, 0.4 cm^3 aliquot was mixed with 0.2 cm^3 each of 1 % sulphanilamide in 3 M HCl and 0.2 % N-naphthylene diamine hydrochloride (NEDH) for recording transmittance at 540 nm. At 105 DAS the plants were harvested to record dry mass of shoots and roots and analyzed for N by Kjeldahl method, Na, K by flame photometry and Ca, Mg by atomic absorption spectrophotometry. N accumulation was calculated as a product of the N content of the plant part and its dry mass.

Results and discussion

Shoot growth: *Rhizobium* inoculation did not increase shoot biomass in non-salinized plants (Table 1) presumably due to nodulation in controls also. However, inoculation had a beneficial effect (+19 %) in the salinity range of 3.3 to 7.4 dS m^{-1}. Although the success of a N_2-fixing symbiotic association is dictated mainly by the tolerance of the host plant (Wilson 1970, Bhardwaj 1975, Velagaletti and Marsh 1989) it may not be the sole determinant because in the present case inoculation with efficient strains improved the growth. At EC 7.4 and 9.2 dS m^{-1} there was very good

vegetative growth initially but declined later on due to salt accumulation. Pillai and Sen (1966) reached similar conclusions with Egyptian clover, although no limits were defined. At high salinity (EC 9.2 dS m^{-1}) chickpea growth at harvest (105 DAS) was very poor and hence inoculation effect was non-significant.

Table 1. Salinity, *Rhizobium* inoculation and nitrate fertilization effects on shoot and root dry mass [g $plant^{-1}$] of chickpea at 105 DAS.

Salinity [dS m^{-1}]	Shoot control	inoculation	nitrate	Root control	inoculation	nitrate
0.8	8.07	8.07	9.17	0.78	0.75	0.76
3.3	6.52	7.37	8.00	0.19	0.20	0.37
5.6	6.00	6.50	7.15	0.19	0.23	0.22
7.4	2.52	3.42	5.97	0.20	0.18	0.24
9.2	1.10	1.20	3.20	0.03	0.02	0.10
CV [%]	2.35			6.60		
CD treatments	0.32			0.05		
CD salinity	0.42			0.07		
CD interactions	0.75			NS		

CV - coefficient of variation; CD - critical difference at $P = 0.05$

The beneficial effect of nitrate was very pronounced at all salinity levels. In non-saline control, increase of plant biomass was 13.6 % and it increased as salinity increased (Table 1). It was 21 % at EC 3.3 - 5.6, 134 % at EC 7.4 and 191 % at EC 9.2 dS m^{-1}. Shoot dry mass declined beyond EC 5.6 dS m^{-1} in uninoculated and inoculated plants and beyond 7.4 dS m^{-1} in nitrate fertilized ones. This clearly proved the alleviating effect of nitrate in helping the plants overcome stress.

Root growth: In contrast to shoots, *Rhizobium* inoculation and/or nitrate fertilization had no significant effect on roots (Table 1) which is contrary to earlier observations on pigeonpea (Rao *et al.* 1994). In the present case shoots provided stronger sink due to initial favorable influences on shoot growth due to inoculation and nitrate fertilization as mentioned earlier. Root biomass decreased drastically at EC 3.3 dS m^{-1} in all treatments.

Nitrate reductase activity: NR activity was reduced at EC 3.3 dS m^{-1} at both growth stages, 70 and 105 DAS (Fig. 1). Both inoculation and nitrate fertilization helped the plants to overcome stress on NR activity at first stage (70 DAS) and reduction occurred only at EC 5.6 dS m^{-1} (Fig 1A). This agrees with the findings of Sharma *et al.* (1989) on mustard. Further, at 105 DAS although NR activity decreased at EC 3.3 dS m^{-1}, yet the decrease was less pronounced. Secondly, inoculation or nitrate did not increase NR activity further except for a nitrate effect in non-saline control (Fig. 1B). This kind of response may be because *a*) elevated NR activity in inoculated and nitrate fertilized plants at earlier stages led to higher leaf N status and reduced need for N and hence lesser enzyme synthesis or *b*) high ion accumulation in

plants at this later stage caused growth disturbance to a degree where inoculation or nitrate fertilization did not make any difference.

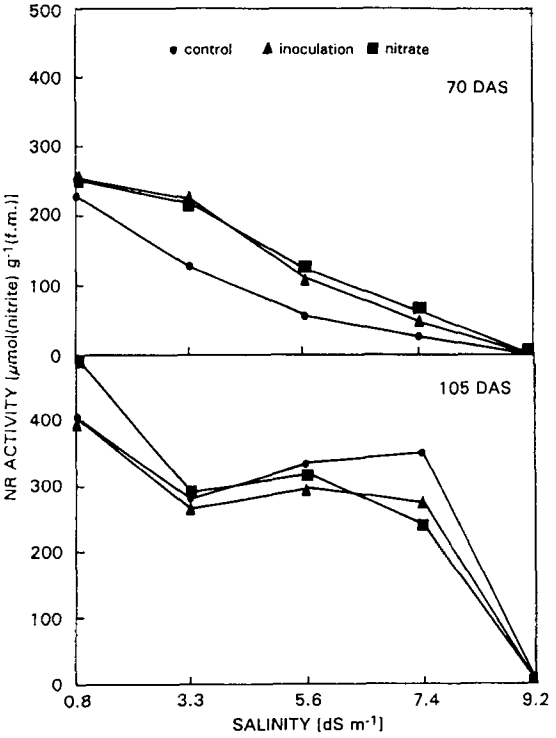

Fig. 1. Alleviation of salinity stress (*circles*) on *in vivo* nitrate reductase activity of chickpea leaves by *Rhizobium* inoculation (*triangles*) or nitrate fertilization (*squares*) at 70 (*A*) and 105 (*B*) DAS.

Nitrogen content and accumulation: Inoculation and nitrate improved N content (% of dry mass) in shoot by 20 and 11 % in non-saline control but not significantly at

Table 2. *Rhizobium* inoculation and nitrate fertilization effects on N accumulation [mg plant^{-1}] in chickpea (105 DAS) under salinity.

Salinity [dS m^{-1}]	Shoot control	inoculation	nitrate	Root control	inoculation	nitrate
0.8	124.9	144.6	147.8	11.5	6.5	13.2
3.3	95.6	113.8	132.4	2.3	2.1	2.4
5.6	94.6	111.0	116.8	2.1	3.7	7.4
7.4	46.6	65.2	101.5	3.9	3.1	4.8
9.2	19.5	19.4	62.2	0.4	0.3	1.9
CV [%]	2.8			10.1		
CD treatments	6.7			1.1		
CD salinity	8.7			1.5		
CD interactions	15.0			2.5		

salinity. There was no effect on roots. There was no significant reduction in N content in shoot or root with increase in salinity which is a result of reduced dry mass production. Total N accumulation decreased with stress and was significant at EC 3.3 dS m^{-1} itself (compared with EC 5.6 for shoot growth) which is a result of the extreme sensitivity of N fixation to salt stress (Wilson 1970, Bhardwaj 1975, Lauter *et al.* 1981, Subba Rao *et al.* 1990). Drastic reduction in N accumulation in shoot and root occurred at EC 7.4 and 3.3 dS m^{-1}, respectively. This decrease is an obvious result of reduced dry mass since N content did not vary significantly. Total N accumulation increased by 11 % on inoculation in non-saline control, 19 % at EC 3.3 and 5.6 and by 35% at EC 7.4 dS m^{-1} proving the beneficial influence of inoculation in protecting symbiotic N fixation from stress effects. Again N accumulation increased to a greater degree in N fed plants, 18 % over non-saline control and 38, 30, 111, 223 % at the EC levels of 3.3, 5.6, 7.4 and 9.2 dS m^{-1}, respectively. Alleviation of stress on N fixation was of the order of 2 EC units with inoculation as well as nitrate supply and like in the case of plant growth, alleviation occurred upto EC 5.6 in inoculated and upto EC 9.2 dS m^{-1} in nitrate fed plants (Table 2).

Accumulation of ions: Imposition of salinity caused increase in Na^+ levels in shoots and roots in control, inoculated and nitrate fed plants. Na^+ was preferentially retained by roots (Table 3), which explains the drastic reduction in root biomass noted earlier. Ca^{2+} content in shoots increased with salinity in all treatments whereas it was stable

Table 3. *Rhizobium* inoculation and nitrate fertilization effects on Na^+/K^+ and Na^+/Ca^{2+} ratios in chickpea (105 DAS) under salinity.

Salinity [dS m^{-1}]	Shoot control	inoculation	nitrate	Root control	inoculation	nitrate
Na^+/K^+						
0.8	0.44	0.35	0.30	1.25	1.67	1.89
3.3	1.75	1.53	1.47	2.32	1.78	1.89
5.6	1.67	1.94	1.51	2.54	2.10	2.11
7.4	1.66	1.93	2.95	3.65	2.24	2.24
9.2	2.57	5.35	2.49	4.76	3.86	3.34
Na^+/Ca^{2+}						
0.8	0.32	0.34	0.26	0.11	0.14	0.11
3.3	0.92	0.88	0.83	0.12	0.13	0.12
5.6	1.16	1.28	1.08	0.15	0.14	0.13
7.4	1.26	1.33	1.53	0.21	0.14	0.12
9.2	1.92	1.77	1.37	0.38	0.22	0.23

in roots. Salinization induced only marginal effects on K^+ contents both in roots and shoots. The ionic accumulation in roots hence protected the shoots from adverse effect of salt injury. Data for individual ion contents is not presented. Sharma (1990) have also shown in chickpea that roots retained more Na^+ than shoot under salinity stress hence affording protection to leaves whereas K^+ concentration of different

plant parts either remained unaffected or increased only slightly. In control plants Na^+ accumulation increased rapidly after EC 5.6 dS m^{-1} in the roots (as evidenced by Na^+/K^+ and Na^+/Ca^{2+} ratios) while similar increase in shoots was noted at EC 7.4 dS m^{-1}. Compared to EC 5.6 dS m^{-1} in control, sharp increases in Na^+/K^+ and Na^+/Ca^{2+} ratios in the roots of inoculated/fertilized plants occurred at EC 7.4 dS m^{-1}. In conclusion, the results indicate that besides inoculation with effective strains of rhizobia, the application of small amounts of fertilizer nitrogen would be beneficial for legume cultivation in saline soils.

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