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Effect of salt stress on interaction between lentil (*Lens culinaris*) genotypes and *Rhizobium* spp. strains: symbiotic N_2 fixation in normal and sodic soils

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Abstract Following screening, selection, characterization and examination of their symbiotic N_2 fixation, only two *Rhizobium* strains (ND-16 and TAL-1860) and four lentil genotypes (DLG-103, LC-50, LC-53 and Sehore 74-3) were found to be suited to sodic soils. Interactions between salt-tolerant lentil genotypes and *Rhizobium* strains were found to be significant, and resulted in greater nodulation, N_2 fixation (nitrogenase activity), total nitrogen, plant height, root length and grain yield in sodic soils under field conditions compared to uninoculated controls. Significantly more nodulation, nitrogenase activity, glutamine synthetase (GS) and NADH-dependent glutamate synthase (NADH-GOGAT) activities were found in normal soil as compared to the soil supplemented with 4% and 8% NaCl. Salt stress inhibited nitrogenase, GS and NADH-GOGAT activities. However, nitrogenase activity in nodules was more sensitive to salt stress than GS and NADH-GOGAT activities (NH_4^+ assimilation). The relevance of these findings for salt-tolerant symbionts is discussed.

Key words *Rhizobium* spp. · Symbiotic N_2 fixation · Nitrogenase activity · Glutamine synthetase · Sodic soils

Introduction

Of the world's 220×10^6 ha of irrigated lands, 40×10^6 ha are affected by salinity, and nearly 40% of the world's land may be susceptible to salinization. Salinity is a major agricultural constraint, and is a significant problem in India because it affects some of the otherwise most productive agriculture areas, including almost 35% of irrigated land in Uttar Pradesh. Increased salt-tolerance of crops has therefore been a major objective of symbiotic N_2-fixation programmes for regions in India where soil salinity is high and water quality is poor. Higher concentrations of NaCl cause marked changes in growth patterns, dry matter allocation, ion transport, water status, physiological processes and biochemical reactions of both partners of symbiotic N_2 fixation (Rai 1992; Cardovilla et al. 1994). Salinity affects the infection process by inhibiting root hair growth and by decreasing the number of nodules per plant and the amount of N_2 fixed per unit weight of nodules. Thus, in saline soils the yield of leguminous crops is decreased due to the lack of successful symbiosis (Hafeez et al. 1988). Salt tolerance in plants is a complex phenomenon that involves morphological and developmental changes, as well as physiological and biochemical processes (Greenway and Munns 1980). Survival and growth in saline environments are the result of adaptive processes, such as ion transport and compartmentation, osmotic solute synthesis and accumulation, which lead to osmotic adjustment and protein turnover for cellular repair (Rai et al. 1985; Munns and Termaat 1986). Excess salts in soil adversely affect survival, growth, nodulation, N_2 fixation and legume-*Rhizobium* symbiosis (Rai et al. 1985; Rai 1987; Bekki et al. 1987; Rai 1992). Studies on root nodules have shown that NH_3 is assimilated in amino acids by the help of the enzymes glutamine synthetase (GS) and the NADH-dependent glutamate synthase (NADH-GOGAT). NaCl in culture media decreased GS and NADH-GOGAT activities in nodules and roots (Bougeais-Chaillou et al. 1992). With increasing world-wide problems of soil salinity and sodicity, the selection of N_2-fixing legumes (pulses) adapted to marginal sodic land deserves to be a high priority of agricultural research.

In the present investigation, suitable lentil (*Lens culinaris*) genotypes and their *Rhizobium* strains were screened in order to select the combinations that produced high yields in normal and salt-affected soils of Uttar Pradesh.

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Materials and methods

Soil used

Normal and sodic soil samples were collected from experimental fields and fertile land of the Instructional Farm of Narendra Deva University of Agriculture and Technology, and analysed for their physical and chemical properties (Jackson 1978). These are presented in Table 1.

Isolation and collection of lentil and *Rhizobium* strains

Thirty six *Rhizobium* strains were isolated from nodules of lentil plants which were collected from salt-affected soils of eastern Uttar Pradesh. Methods described by Vincent (1970) were used for characterization, employing yeast extract mannitol medium. The isolates were inoculated on N-free Burk's medium to check for contamination by free N_2 fixers, and characterized on the basis of morphological, biochemical and plant infection tests (Vincent 1970). Thirty-three *Rhizobium* strains of lentil were also obtained from National and International centres, especially Indian Agricultural Research Institute, New Delhi, Nitrogen fixation by Tropical Agricultural Legumes (Nif-TAL) and USDA. Out of 69 strains, four (ND-16, TAL-1860, TAL-1235 and TAL-51) were selected for further study. Lentil *Rhizobium* strain ND-16 was isolated from eastern Uttar Pradesh and TAL-1860, TAL-1235 and TAL-51 were obtained from Nif-TAL.

Screening of lentil genotypes/germplasm tolerant to sodic soil (field experiment 1, 1993–1994)

A total of 1388 genotypes/germplasms were tested for germination, growth, and nodulation in sodic soil (data not presented) of which the best 146 genotypes were selected for further experimentation.

Screening of lentil genotypes tolerant to sodic soil (field experiment 2, 1994–95)

The 146 lentil genotypes selected between 1993 and 1994 were again screened for tolerance to sodic soil with respect to germination, growth, nodulation (number of nodules/plant and their dry weight), plant height, root length, shoot dry weight and grain yield. Only 12 genotypes, DLG-103, DLG-132, L-227, B-183, L-281/83, SL-338, LC-50, LC-53, LC-194, LC-383, DLG-66 and Sehore 74-3 were found to be suitable for sodic soils. However, only four genotypes, DLG-103, LC-50, LC-53 and Sehore 74-3, were found to be the most suitable for the sodic and normal soils, and were used for further experiments.

Table 1 Chemico-physical properties of sodic and normal soils used in the study. *Ec* Electrical conductivity

Soil properties	Sodic soil	Normal soil
pH (1:25 soil:water)	9.63	7.20
Ec (1:25 soil:water) (S m^{-1})	0.39	0.15
Exchangeable Na (%)	22.50	2.50
Organic C (%)	0.21	0.44
Exchangeable Ca (%)	2.05	40.1
Available N (kg ha^{-1})	112.3	200.0
Available P (kg ha^{-1})	13.6	21.95
Available K (kg ha^{-1})	187.0	300.93
Available Fe (mg kg^{-1})	3.27	15.05
Available Zn (mg kg^{-1})	0.37	0.85
Sand (%)	77.40	53.20
Silt (%)	7.60	35.50
Clay (%)	15.0	11.30
Textural class	Sandy loam	Sandy loam

Effect of pH on growth of *Rhizobium* strains

The growth of 66 isolates of *Rhizobium* was studied for 9 days at 28 °C at different pH levels (pH 6.0, 7.0, 8.0, 9.0, 10.0) using yeast extract mannitol liquid medium. The desired pH of the medium was maintained during the growth of the strains by 1.0 N HCl and NaOH. Growth was measured as optical density at 625 nm. Out of the 66 isolates, only ND-16 and TAL-1860 were found to be fast growing; TAL-1235 and TAL-51 were noted as slow growing at higher pH levels and thus these four strains were selected for further studies.

Effect of Na salts on growth of *Rhizobium* strains

The effects of NaCl, $MgSO_4 \cdot 7H_2O$, Na_2CO_3 and $NaHCO_3$ (0–8%) on the growth of *Rhizobium* strains TAL-1860, ND-16, TAL-1235 and TAL-51 were assessed in yeast extract mannitol liquid medium. Cultures were grown for 9 days at 28 °C with the respective salt (five replicates) and the pH of the medium was maintained at 8.5 by the regular addition of 1.0 N NaOH.

pH maintenance

To determine the desired pH of the medium, experiments were conducted with two sets of yeast extract mannitol liquid medium (five replicates per set). One set was used to measure the pH by pH meter at regular intervals; a known volume of 1.0 N NaOH was added to maintain the pH, and the set was then discarded because of the possibility of contamination. In the other set, the same volume of 1.0 N NaOH was added to maintain the desired pH (8.5).

Survival and growth of *Rhizobium* strains in sodic and normal soils

Rhizobium strains. TAL-1860, ND-16, TAL-1235 and TAL-51 were tested for survival and growth in sterilized sodic and normal soils (Hartle and Alexander 1983), and the population sizes were determined thereafter by dilution and platecounting techniques.

Effect of interactions between salt-tolerant lentil genotypes and *Rhizobium* strains on nodulation, nitrogenase activity and other plant characteristics in sodic soil 45 days after sowing (field experiment 3, 1995–96)

The effects of interactions between salt-tolerant lentil genotypes (DLG-103, LC-50, LC-53 and Sehore 74-3) and *Rhizobium* strains (TAL-1860, ND-16 and uninoculated control) on nodulation, nitrogenase activity, total N accumulation, plant height and root length were assessed under field conditions. The field experiment was conducted in a split-plot design with three replicates of plot size 4×1.8 m. Strains (*Rhizobium*) and genotypes (lentil) were used in main and sub-plots respectively. Seeds of the lentil genotypes were slurry inoculated with *Rhizobium* strains and sown in plots at a spacing of 30×10 cm with 240 plants per plot. The survival of *Rhizobium* strains on different seeds was assessed and noted more than 10^6 viable cells per seed, autoclaved cell suspensions of strains were also used in the uninoculated treatments. Fertilizers were applied uniformly in plots at rates of 20 kg N ha^{-1}, 45 kg P ha^{-1}, 50 kg K ha^{-1} and 2 kg Zn ha^{-1} in the form of urea, single superphosphate, KCl and $ZnSO_4$.

Forty-five days after sowing, nodule numbers and dry weights, plant dry weights, shoot and root dry weight, plant height, root length and nitrogenase activity (Rai 1987) were measured. At maturity, 230 plants from each plot were harvested to record the grain yield (12% moisture content).

Pot culture experiment 1 (1995–1996)

To evaluate the effect of strains TAL-1860 and ND-16 on symbiotic N_2 fixation, nodulation, nitrogenase activity, GS and NADH-GOGAT activity of nodules, and soluble proteins of fresh nodules of salt-tolerant lentil genotypes (DLG-103, LC-50, LC-53 and Sehore 74-3) with different concentrations of applied NaCl (0, 4, 8%) in normal soil, a pot culture experiment was conducted in the third week of October, using 15 kg normal soil per pot. Five replicates for each strain, genotype and NaCl level were used in a completely randomized block design. Contamination was successfully avoided during the experiment. For microbiological control, seeds of salt-tolerant genotypes were surface-sterilized with 90% ethanol and mercuric chloride for 5 min and 3 min, respectively, followed by ten rinses in sterile water. The surface-sterilized seeds were inoculated with each salt-tolerant strain, using 2 ml broth containing approximately 6×10^6 cells ml^{-1}.

The inoculum level was assessed at the time of sowing as 10^7 cells per seed. Autoclaved cell suspensions of strains were used in the uninoculated treatments and five plants were raised in each pot. Plants were grown in a glasshouse and watered when needed. N (12.5 mg kg^{-1} soil), P (20.0 mg as P_2O_5 kg^{-1} soil) and K (10 mg K_2O kg^{-1} soil) were added in the form of urea, single superphosphate and KCl, respectively. Zn, Mo and Fe were also added at the rate of 5 mg kg^{-1} soil in the form of $ZnSO_4$, $NaMoO_4$ and $FeSO_4$.

Forty-five days after sowing, nodule numbers and dry weights, nitrogenase activity (Rai 1987), proteins of fresh nodules, GS and NADH-GOGAT activities in nodule homogenates were determined according to the modified techniques of Groat and Vance (1981).

Nitrogenase activity

The nitrogenase activity of fresh nodules was determined using the acetylene reduction assay in a continuous flow system (Rai 1987), with 12% acetylene in air at a rate of 100 ml min^{-1}. Samples were taken after 1 h of incubation. Acetylene and ethylene were determined by an Amil-Nucon-44 gas chromatograph equipped with a hydrogen flame ionization detector and a 50-cm stainless steel column (0.23 cm external diameter) filled with poropak N (90–100 mesh), operated at 140 °C with the carrier gas (N_2) at a flow rate of 40 ml min^{-1}.

Preparation of cell-free extracts for GS, NADH-GOGAT and protein

Nodule homogenates for determining GS, NADH-GOGAT and protein were prepared according to the methods adapted by Groat and Vance (1981). The homogenate was filtered through four layers of cheescloth, and nodule debris removed. The filtrate was centrifuged at 3500 rpm at 2 °C for 20 min, producing a clear solution of host-cell cytoplasm and its organelles; this solution was used for enzyme assays.

Enzyme assays

GS activity was measured by the hydroxamate synthetase assay as used by Farden and Robertson (1980) and Kaiser and Lewis (1984). Two controls, one without enzyme and one without L-glutamate were also analysed. NADH-GOGAT activity was determined spectrophotometrically at 30 °C by monitoring the oxidation of NADH at 340 nm as suggested by Groat and Vance (1981). Two controls (without α-ketoglutorate and without glutamine) were used to correct for endogenous NADH oxidation. Protein in tissue extracts was determined by the Bradford method (1976) with bovine serum albumin as the standard.

Statistical analysis

The results were subjected to two-way ANOVA with the least significant difference test between means.

Results

Growth and survival of *Rhizobium* strains in sodic and normal soils

Out of 60 lentil *Rhizobium* isolates, only two strains proved suitable for nodulation and growth in sodic and normal soils. Changes in the populations of four strains of lentil 10, 20 and 30 days after inoculation are presented in Table 2. The count was highest for strain ND-16 (22.9×10^6 g^{-1} soil) followed by strain TAL-1860 (20.6×10^6 g^{-1} soil) in sodic soil. Significant growth and survival of other strains such as TAL-1235 and TAL-51 were also recorded in normal soil, but these two strains did not survive in sodic soil. However, the maximum counts of strains ND-16 and TAL-1860 were also recorded in normal soil as compared to sodic soil.

Table 2 Growth and survival of lentil *Rhizobium* strains in sterilized sodic and normal soils. Means of five replicate cultures after 10, 20 and 30 days of growth. *LSD* Least significant difference

Strains	Cell count $\times 10^6$ g^{-1} soil					
	Normal soil			Sodic soil		
	10 days	20 days	30 days	10 days	20 days	30 days
TAL-1860	13.2	16.9	24.6	8.5	13.2	20.6
ND-16	14.1	17.5	24.8	9.7	14.1	22.9
TAL-1235	11.2	14.3	19.9	0.2	0.3	0.3
TAL-51	10.1	12.5	16.7	0.01	0.05	0.07
LSD ($P = 0.05$)	0.39	0.76	0.06	0.01	0.08	0.07

Effect of pH on growth of *Rhizobium* strains

The growth data of sodic-soil-tolerant *Rhizobium* strains (TAL-1860 and ND-16) in yeast extract mannitol medium at different pH levels (pH 6.0, 7.0, 8.0, 9.0 and 10.0) are presented in Table 3. Increasing or decreasing pH levels, except neutral pH (pH 7.0), inhibited the growth of *Rhizobium* strains. However, strains TAL-1860 and ND-16 showed significantly better growth at higher pH levels and tolerated a pH of up to 10.0. Strains TAL-1235 and TAL-51 showed very poor growth at pH 9.0 and 10.0.

Table 3 Effect of pH on the growth [optical density (OD) at 625 nm] of *Rhizobium* strains in yeast extract mannitol (YEM) medium

Strains	pH				
	6	7	8	9	10
TAL-1860	0.303	0.859	0.732	0.504	0.247
ND-16	0.282	0.848	0.716	0.589	0.232
TAL-1235	0.153	0.460	0.332	0.187	0.097
TAL-51	0.095	0.294	0.167	0.093	0.023
LSD ($P=0.05$)	0.019	0.032	0.012	0.020	0.013

Effect of NaCl, $MgSO_4 \cdot 7H_2O$, Na_2CO_3 and $NaHCO_3$ on growth of *Rhizobium* strains

The growth of *Rhizobium* strains TAL-1860, ND-16, TAL-1235 and TAL-51 was studied with different concentrations of NaCl (0–8%), $MgSO_4$ (0–4%), Na_2CO_3 (0–2%) and $NaHCO_3$ (0–2%) in yeast extract mannitol medium at pH 8.5 (Fig. 1). Increasing concentrations of different salts resulted in decreasing growth of *Rhizobium* strains. Among the salts tested, HCO_3^- was found to be the most toxic to the strains, followed by CO_3^-, SO_4^{2-} and Cl^-.

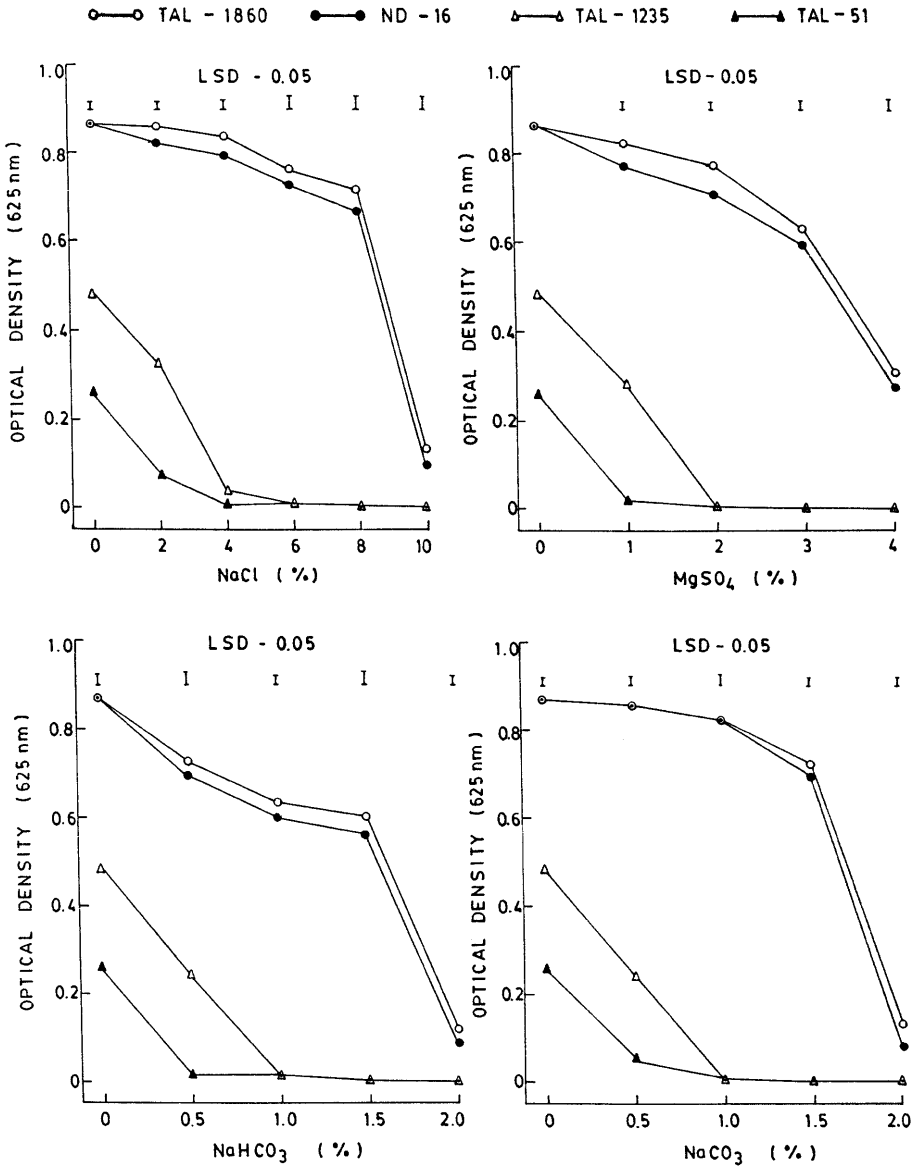

Fig. 1 Effect of salts (NaCl, $MgSO_4$, $NaHCO_3$ and $NaCO_3$) on growth (optical density at 625 nm) of lentil *Rhizobium* strains TAL-1860, ND-16, TAL-1235, and TAL-51. *LSD* Least significant difference

Combined effect of NaCl, $MgSO_4 \cdot 7H_2O$, Na_2CO_3 and $NaHCO_3$ on growth of *Rhizobium* strains

The combined effect of NaCl, $MgSO_4 \cdot 7H_2O$, Na_2CO_3 and $NaHCO_3$ on the growth of *Rhizobium* strains was studied at pH 8.5, and the results are presented in Table 4. The observations revealed that strains TAL-1860, and ND-16 showed tolerance up to 4.0% NaCl plus 2.0% $MgSO_4 \cdot 7H_2O$ plus 0.75% Na_2CO_3 plus 0.75% $NaHCO_3$. Increasing concentrations of these salts (6.0% NaCl plus 3.0% $MgSO_4 \cdot 7H_2O$ plus 0.75% Na_2CO_3 plus 0.75% $NaHCO_3$) completely inhibited the growth of strains TAL-1860 and ND-16. The strains showed resonable growth at all concentrations of the four salts, but responded differently to different concentrations of Cl^{-1}, SO_4^{2-}, CO_3^- and HCO_3^-. Better growth was observed with strain TAL-1860 followed by ND-16 at different concentrations of the various salts. Moreover, increasing salinity decreased the growth of all strains. The responses to NaCl plus $MgSO_4 \cdot 7H_2O$ plus Na_2CO_3 plus $NaHCO_3$, and their different combinations in terms of the growth of the two strains were significant, and resulted in significantly poorer growth (Table 4).

Effect of interactions between strains and genotypes on nodulation, nitrogenase activity and other plant characteristics in sodic soil

The effects of salt-tolerant *Rhizobium* strains on the numbers and dry weight of nodules, nitrogenase activity, total plant N, plant height and root length of the four salt-tolerant genotypes of lentil in sodic soil in a field experiment are shown in Table 5. A significant increase in the number and dry weight of nodules, nitrogenase activity, total N accumulation in plant, plant height and root length were produced by the symbiosis between genotype Sehore 74-3 and strain TAL-1860, followed by those between Sehore 74-3 and ND-16, and LC-53 and TAL-1860. Strains differed significantly with respect to nodulation and symbiotic N_2 fixation, although there were differences among genotypes. Among the lentil genotypes, Sehore 74-3 showed a significantly higher nodule number, nitrogenase activity total N accumulation, plant height and root length than the others.

Effects of interactions between salt-tolerant lentil genotypes and *Rhizobium* strains on grain yield (field experiment 3, 1995–1996)

Data on the effects of strains and genotypes on grain yield (Table 6) indicated that the interaction between *Rhizobium* strains and genotypes gave significantly higher grain yields. Strains TAL-1860 and ND-16 produced a significantly higher grain yield than the native *Rhizobium* strain. Strain TAL-1860 significantly increased the yield of genotypes Sehore 74-3, LC-50, LC-53 and DLG-103 compared to ND-16. Irrespective of the strains, genotype Shore 74-3 gave the highest grain yield.

Effects of interactions among *Rhizobium* strains, lentil genotypes and NaCl levels on nitrogenase activity, GS activity, NADH-GOGAT activity, and proteins of nodules in normal soil

The effects of salt-tolerant *Rhizobium* strains on nitrogenases activity, GS activity, NADH-GOGAT activity, and proteins of fresh nodules of the four salt-tolerant genotypes of lentil at two levels of applied NaCl (4% and 8%) in normal soil are shown in Tables 7 and 8. Differences among salt-tolerant strains were evident from the nitrogenase activities observed at different levels of applied NaCl. The highest level of nitrogenase activity was observed at 0% NaCl, whereas 4% and 8% NaCl reduced or inhibited nitrogenase activity significantly compared to GS activity and NADH-GOGAT activities. However, the effect of salinity on the acetylene-reduction activity of nodules was more pronounced than its effect on GS and NADH-GOGAT activities of nodules. The mean inhibition of nitrogenase activity for the two NaCl levels was 49% and 61%, re-

Table 4 Combined effect of NaCl, $MgSO_4 \cdot 7H_2O$, Na_2CO_3 and $NaHCO_3$ on the growth (OD) at 625 nm of *Rhizobium* strains at pH 8.5 in YEM medium. For abbreviations, see Tables 2 and 3

Treatments	*Rhizobium* strains			
	TAL-1860	ND-16	TAL-1235	TAL-51
Control	0.871	0.870	0.495	0.280
2% NaCl + 1% $MgSO_4 \cdot 7H_2O$ + 0.50% Na_2CO_3 + 0.50% $NaHCO_3$	0.726	0.711	0	0
4% NaCl + 2% $MgSO_4 \cdot 7H_2O$ + 0.75% Na_2CO_3 + 0.75% $NaHCO_3$	0.539	0.521	0	0
6% NaCl + 3% $MgSO_4 \cdot 7H_2O$ + 1% $NaCO_3$ + 1% $NaHCO_3$	0	0	0	0
LSD ($P = 0.05$)	0.029	0.045	0.013	0.016

Table 5 Effect of interations between salt-tolerant lentil genotypes (*DLG-103, LC-50, LC-53, Sehore 74-3*) and salt-tolerant *Rhizobium* strains (*TAL-1860, ND-16*) on nodulation, nitrogenase (*N_2ase*) activity, total N accumulation, plant height and root length in salt-affected soil 45 days after sowing

Treatments	No. of nodules plant $^{-1}$	Dry weight of nodules plant $^{-1}$ (mg)	N_2ase activity (μmol C_2H_4 g $^{-1}$ fresh nodules h $^{-1}$)	Total N (mg plant $^{-1}$)	Plant height (cm)	Root length (cm)
DLG-103 (uninoculated)	11.50	8.32	11.05	5.89	17.8	9.8
DLG-103 × TAL-1860	28.41	20.45	54.19	12.20	22.1	12.6
DLG-103 × ND-16	26.00	17.64	50.13	11.70	17.4	10.8
LC-50 (uninoculated)	10.16	8.00	10.93	5.06	16.2	8.2
LC-50 × TAL-1860	22.58	19.36	59.30	15.50	25.4	13.5
LC-50 × ND-16	24.41	16.07	56.39	13.80	23.1	14.7
LC-53 (uninoculated)	8.32	7.24	10.95	5.52	17.8	10.6
LC-53 × TAL-1860	21.60	21.17	62.22	16.60	26.6	18.7
LC-53 × ND-16	23.38	19.93	58.18	12.50	22.0	17.3
Sehore 74-3 (uninoculated)	12.20	8.95	10.98	5.70	20.5	12.2
Sehore 74-3 × TAL-1860	27.81	27.92	72.20	19.25	28.8	20.6
Sehore 74-3 × ND-16	28.00	25.11	69.35	18.05	25.9	19.4
LSD ($P = 0.05$)						
Strain	1.38	0.72	4.12	0.41	1.05	0.98
Genotype	2.58	1.06	8.25	0.50	2.18	1.53
Strain × genotypes	3.32	2.01	10.81	0.85	3.97	3.75

Table 6 Effect of interaction between salt-tolerant lentil genotypes and salt-tolerant *Rhizobium* strains on grain yield under salt-affected soil

Lentil genotypes	Control (uninoculated)	Grain yield (kg ha $^{-1}$)	
		TAL-1860	ND-16
		Rhizobium strains	
DLG-103	1293	1584	1451
LC-50	1377	1738	1606
LC-53	1331	1698	1543
Sehore 74-3	1531	1897	1754
LSD ($P = 0.05$)			
Strain		142	
Genotype		275	
Strain × genotype		306	

spectively. Salinity also reduced the activities of both GS and NADH-GOGAT in nodules, i.e. in relation to the concentration of NaCl present in the soil. The inhibitory effect of NaCl was more pronounced in the case of NADH-GOGAT activity than GS activity (Tables 7, 8). The protein content of the nodules was significantly inhbited by increasing concentrations of NaCl, as compared with the controls, although the effect of NaCl on the protein content was less marked than its effect on ARA, GS and NADH-GOGAT activities in nodules.

Discussion

In legumes, salt stress significantly limits productivity because of its adverse effects on the growth of the host plant and root nodule bacteria, symbiotic development and N-fixation efficiency (Rai 1992; Cardovilla et al. 1994). Salt-tolerant lentil genotypes and their *Rhizobium* strains were isolated and selected for their ability to effectively grow and fix N_2 under salt-stress conditions in sodic soil. The effect of various salts on the growth of different strains was variable, and it appeared that, like most of *Rhizobium* strains, lentil *Rhizobium* strains TAL-1860 and ND-16 were sensitive to higher concentrations of salts and various combinations of Na and Mg salts at different concentrations (Rai 1987, 1992). Genetic determinants in TAL-1860 and ND-16 might have been responsible for the better salt tolerance of these strains, resulting in more growth, greater populations, and effective symbiotic N_2 fixation with salt-tolerant lentil genotypes in sodic soil (Tables 2, 4, 5). The mechanism of salt-tolerance in lentil genotypes is not known; their longer roots, such as those of genotype Sahore 74-3 followed by LC-53 (Table 5), that penetrate deeper into the soil may be an "escape" mechanism, as salts frequently rise upwards in soil because of capillarity in the winter season. Other physiological mechanisms cannot be ruled out. However, their genetically determined mechanism of salt tolerance seemed to help the *Rhizobium* strains to thrive, compete and perform better in symbiosis with salt-tolerant genotypes at higher levels of applied NaCl in normal soil (Tables 7, 8). Symbiotic N fixation within the nodule is only the culmination of a long chain of events involving the recognition and penetration of root hairs, the induction of infection threads and nodule development, and morphogenetic changes during the formation of the bacteroid. Most of these steps are known to be controlled by genes both in the host and bacterium (Rai and Singh 1979).

Further, the two selected strains (TAL-1860 and ND-16) which showed equal salt tolerance, produced

Table 7 Effect of interactions between lentil genotypes, *Rhizobium* strains and NaCl on N_2ase activity and glutamate synthetase (*GS*) activity of nodules in normal soil after 45 days of growth. For other abbreviations, see Tables 2 and 5

Treatments	N_2ase activity (μmol C_2H_4 g^{-1} fresh nodule h^{-1}) NaCl (%)			GS activity (μmol γ-glutamyl-hydroxamate fresh nodule h^{-1}) NaCl (%)		
	0	4	8	0	4	8
DLG-103 (uninoculated)	10.50	4.73	1.37	256.4	219.4	190.2
DLG-103 × TAL-1860	53.25	32.72	23.17	260.3	220.9	197.6
DLG-103 × ND-16	50.31	28.69	21.29	262.2	218.5	197.3
LC-50 (uninoculated)	12.39	5.73	1.36	268.4	207.2	185.3
LC-50 × TAL-1860	58.19	36.43	24.37	265.8	213.1	188.9
LC-50 × ND-16	54.22	34.49	22.33	262.6	219.4	188.2
LC-53 (uninoculated)	15.37	9.29	2.15	265.4	211.5	189.7
LC-53 × TAL-1860	60.48	42.53	28.17	262.7	218.8	187.6
LC-53 × ND-16	58.56	40.59	26.29	265.3	213.9	188.8
Sehore 74-3 (uninoculated)	19.74	11.27	3.94	290.9	232.1	201.0
Sehore 74-3 × TAL-1860	68.43	46.17	33.23	293.1	238.6	208.7
Sehore 74-3 × ND-16	65.57	42.23	30.27	294.7	242.8	205.2
LSD ($P = 0.05$)						
Strain	1.17	1.16	1.12	4.5	2.5	3.7
Genotype	2.27	1.26	1.29	5.2	3.5	4.2
NaCl	2.54	2.52	2.49	6.3	5.7	5.4
Strain × genotype	3.86	3.89	2.87	7.1	6.4	8.8
Genotype × NaCl	4.05	4.97	3.47	8.8	8.9	9.5
Genotype × NaCl × strain	5.62	5.72	5.29	8.7	10.2	11.3

Table 8 Effect of interactions between lentil genotypes. *Rhizobium* strains and NaCl on NADH-dependent glutamate synthase (*NADH-GOGAT*) activity and protein of nodules in normal soil after 45 days of growth. *NADHox*·NADH oxidation

Treatments	NADH-GOGAT activity (μmol NADHox g^{-1} fresh nodule h^{-1}) NaCl (%)			Protein (mg g^{-1} fresh nodule) NaCl (%)		
	0	4	8	0	4	8
DLG-103 (uninoculated)	67.13	57.35	48.44	6.70	6.45	4.29
DLG-103 × TAL-1860	68.92	52.29	45.71	7.32	6.37	5.45
DLG-103 × ND-16	68.73	55.15	46.29	7.51	6.15	5.14
LC-50 (uninoculated)	61.94	50.28	40.41	6.94	5.83	4.48
LC-50 × TAL-1860	63.76	50.21	42.29	7.86	6.72	6.70
LC-50 × ND-16	58.41	49.50	42.27	7.50	6.95	6.04
LC-53 (uninoculated)	62.30	52.28	42.19	7.42	6.37	5.36
LC-53 × TAL-1860	63.72	53.27	43.75	8.48	7.85	6.51
LC-53 × ND-16	65.43	54.51	44.29	8.79	7.80	6.31
Sehore 74-3 (uninoculated)	70.72	62.29	48.49	11.28	8.48	6.29
Sehore 74-3 × TAL-1860	75.83	65.78	52.92	11.75	9.73	7.21
Sehore 74-3 × ND-16	72.20	62.28	50.19	11.49	9.64	7.16
LSD ($P = 0.05$)						
Strain	1.29	1.53	1.61	0.72	0.63	0.67
Genotype	1.36	1.42	1.51	0.48	0.73	0.78
NaCl	1.85	1.75	1.93	0.97	0.99	0.97
Strain × genotype	2.11	2.19	2.15	1.18	1.19	1.21
Genotype × NaCl	3.43	3.55	3.29	1.97	1.88	1.97
Genotype × NaCl × strain	4.97	5.06	5.17	2.01	2.22	2.35

variable patterns of nodulation, and other characteristics which affected yield (Tables 5–8) may have been due to the activities of host genes and/or to the *Rhizobium* strains and NaCl concentrations applied (Tables 7, 8). The differential interactions observed between genotypes and strains, as affected by various levels of applied NaCl, were quantitiative rather than qualitative, and each variable considered in this investigation can be controlled by a polygenic system. Selected cultivars which benefitted from inoculation with the efficient and effective *Rhizobium* strains TAL-1860 and ND-16 also responded significantly to applied NaCl.

In root nodules, fixed N in the form of NH_3, is exported from bacteroids into the host-plant cytoplasm, where further assimilation into amino acids occurs by the catalytic action of GS and NADH-GOGAT (Groat and Vance 1981). In their studies of soybean, Bougeais-Chaillou et al. (1992) reported that NaCl in the culture medium decreased GS and NADH-GOGAT activities in nodules. With increasing world-wide problems of soil salinity, the selection and identification of N_2-fixing legumes for use in marginal saline and sodic lands deserves to be a high priority of agricultural research. Similar results to those of Bougeais-Chaillou et al. (1992) and Cardovilla et al. (1994) were obtained in our work with salt-tolerant lentil genotypes and their *Rhizobium* strains. Salinity affected nitrogenase activity more than GS and NADH-GOGAT activities. Salt stress inhibited acetylene reduction to a greater extent than GS activity. In plants grown without NaCl, GS activity was found to be double that of NADH-GOGAT activity. Treatment with 4% NaCl affected GS more than NADH-GOGAT activities, whereas the activities of both enzymes were significantly inhibited by 8% NaCl, a finding in conformity with that of Billard and Boucaud (1980). The decrease in the protein content of the nodules is a typical response to salt stress, and has also been seen in other legumes (Bougeais-Chaillou et al. 1992). This response might have been due to protein breakdown or to an alteration in the incorporation of amino acids into proteins (Stewart and Lee 1979). In this connection, the selective accumulation of intracellular amino acids is a prominent physiological response of many organisms to osmotic stress (Yancey et al. 1982). Under certain conditions the GS-GOGAT pathway is thus of great importance in determining the expression of *nif* genes (Dixon and Cannon 1976). GS appears to act as a positive inducer of nitrogenase, and *nif* genes are controlled by GS in the same manner as GS the positive control element for nitrogenase synthesis (Johnston and Beringer 1977). The control of enzyme synthesis is exerted by activating the transcription of operons whose gene products are involved in the utilization of a N source by the biosynthetically active GS enzyme. The operon for N_2 fixation remains repressed in *Rhizobium,* and the host plant provides the triggering factor (activator) resulting in repression of the *nif* operon. Saline-tolerant strains may be constitutive and there might have been an altered nitrogenase which may be resistance to a high salt concentration. Work on this problem is still in progress. At least 13 different multigene systems (stimulants) are known to be induced in response to a variety of stress stimuli such as heat, cold and salt shock (Van Bogelen et al. 1990). The proteins encoded by these stress genes have to cope with unfavourable situations to ensure survival under stress conditions. These osmoprotectant proteins reduce inhibitory effects of high osmolarity and promote growth and symbiotic N_2 fixation under salt stress.

The obvious significance of this work in agriculture is in the development of *Rhizobium* strains immune to adverse soil conditions in order to help rhizobia-inoculated legumes to establish under such conditions, and thus promote successful symbiosis and higher yields of grain legumes. To achieve successful crops under sodic soils, simultaneous selection of saline-tolerant symbionts is necessary. In this study, the symbiosis of *Rhizobium* strain TAL-1860 and lentil genotype Sehore 74-3 was found to be the most successful under saline conditions.

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