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Response of the pigeonpea-*Rhizobium* symbiosis to salinity stress: Variation among *Rhizobium* strains in symbiotic ability *

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Summary. There were significant differences among pigeonpea [*Cajanus cajan* (L.) Millsp] *Rhizobium* sp. strains (IC 3506, IC 3484, IC 3195, and IC 3087) in their ability to nodulate and fix N_2 under saline conditions. Pigeonpea plants inoculated with IC 3087 and IC 3506 were less affected in growth by salinity levels of 6 and 8 dS m^{-1} than plants inoculated with the other strains. For IC 3506, IC 3484, and IC 3195, there was a decrease in the number of nodules with increasing salinity, while the average nodule dry weight and the specific nitrogenase activity remained unaffected. However, in IC 3087, the number of nodules increased slightly with increasing salinity. Leaf-P concentrations increased with salinity in the inoculated plants irrespective of the *Rhizobium* sp. strain, and leaf-N concentrations decreased with increasing salinity in IC 3484 and IC 3195 only. Shoot-Na and -Cl levels were further increased in these salt-sensitive strains only at 8 dS m^{-1}. Therefore there may be scope for selecting pigeonpea *Rhizobium* sp. symbioses better adapted to saline conditions. The *Rhizobium* sp. strains best able to form effective symbioses at high salinity levels are not necessarily derived from saline soils.

Key words: *Cajanus cajan* – Pigeonpea – *Rhizobium* variation – Salinity stress – Symbiotic nitrogen fixation

Pigeonpea [*Cajanus cajan* (L.) Millsp] is one of the important grain legumes grown in semi-arid regions where salinity problems can be acute (Chauhan 1987). Since the agricultural importance of legumes is particularly related to their ability to fix N_2 in root nodules, any study on the feasibility of growing pigeonpea under saline conditions must include information on the effects of salinity on the pigeonpea–*Rhizobium* sp. symbiosis. Various reports have been published on salinity effects on the nodulation and N_2 fixation of different legumes (Bernstein and Ogata 1966; Wilson 1970; Lakshmi-Kumari et al. 1974; Balasubramanian and Sinha 1976; Lauter et al. 1981; Yousef and Sprent 1983; Singleton and Bohlool 1984; Siddiqui et al. 1985) but only a single *Rhizobium* sp. strain was used in each study. There is wide variation among *Rhizobium* sp. strains in their ability to grow and survive under saline conditions in yeast extract mannitol agar medium (Singleton et al. 1982; Subbarao 1984); the extent of the variation in their symbiotic ability under saline conditions is still to be determined. In the present investigation, we attempted to measure this variation in the pigeonpea-*Rhizobium* sp. symbiosis.

Materials and methods

Rhizobium sp. strains and culture conditions. The *Rhizobium* sp. cultures used in the present study were obtained from the pigeonpea *Rhizobium* collection of the Legumes Program, ICRISAT, India. These were selected on the basis of their growth habit, tolerance to NaCl on yeast extract mannitol agar, and ecological origin (Table 1), and were all effective in fixing N_2 in symbiosis with pigeonpea. All cultures were maintained on yeast extract mannitol agar slopes (Vincent 1970). A loopful of *Rhizobium* sp. culture was inoculated into the sterilized yeast extract mannitol broth and incubated at 28 °C for 3 and 7 days for fast- and slow-growing strains, respectively. After incubation, the culture was checked for purity by streaking a loopful of the broth culture on congo red yeast extract mannitol agar and incubating at 28 °C.

Plant culture. The growth medium consisted of sieved river sand, which was washed and soaked in acid solution (pH 1–2) for 24 h, and then thoroughly washed with tap water, dried, and filled in 180 mm diameter plastic pots. The pots with sand were steam-sterilized. Pigeonpea seeds of the salinity-tolerant genotype ICPL 227 were surface-sterilized with 0.2% $HgCl_2$ solution for 5 min and

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Table 1. Origin and growth characteristics and tolerance to NaCl salinity on yeast extract mannitol agar (YEMA) of pigeonpea *Rhizobium* cultures used in the study

Rhizobium	Strain origin	Soil isolated from	Growth on YEMA	Tolerance to NaCl on YEMA ($dS\ m^{-1}$)
IC 3506	Gujarat, India	Vertisol	Fast	86.0
IC 3484	Maharastra, India	Vertisol	Slow	34.2
IC 3087	ICRISAT Center	Vertisol (saline)	Slow	8.6
IC 3195	ICRISAT Center	Alfisol	Slow	4.3

then washed with deionized water. Eight surface-sterilized seeds were sown in each pot. The sand surface in each pot was covered with 50 g of polythene beads in order to minimize evaporation, and the pots were supplied with sterilized deionized water. For all watering and flushing operations, the solutions were sterilized before use in order to avoid cross contamination. On the 10th day after sowing, the seedlings were thinned to four per pot. There were four salinity treatments, 0, 4, 6, 8 dS m^{-1}, and seven N/*Rhizobium* sp. treatments: (1) fed with inorganic N; (2) inoculated with *Rhizobium* sp. strain IC 3024 at the time of sowing (IC 3024 early); (3) inoculated with IC 3024 14 days after sowing (IC 3024 late); (4) IC 3506 late; (5) IC 3484 late; (6) IC 3087 late; and (7) IC 3195 late. The N-fed treatment was given 50 mg l^{-1} N as NH_4NO_3 from 28 days after sowing. The results of the N-fed, and the IC 3024 early and late treatments are being published separately.

A modified Arnon and Hoagland N-free nutrient solution (1 : 4 strength) amended with NaCl+$CaCl_2$ (1 : 1 w : w) was used for the various salinity treatments. The composition of the nutrient solution (full strength) in *mM* was: 0.9 KH_2PO_4, 2.08 KCl, 1.01 $MgSO_4 \cdot 7H_2O$, 1.46 $CaCl_2 \cdot 2H_2O$, 0.006 $MnSO_4 \cdot H_2O$, 0.0009 $ZnSO_4 \cdot 7H_2O$, 0.001 $CuSO_4 \cdot 5H_2O$, 0.004 H_3BO_3, 0.0002 $Na_2MoO_4 \cdot 2H_2O$, 0.16 NaFeEDTA. The electrical conductivity of the nutrient solution (1 : 4 strength) without any added salt was 0.33 dS m^{-1}. The experiment was arranged in a randomized block design with four replications, in a greenhouse with a mean temperature of 28/22 °C (day/night) and relative humidity of 60–70% (mean day+night).

The salinity treatments were imposed on the 14th day after sowing by flushing each pot with 1 l treatment solution and the *Rhizobium* sp. inoculation was done afterwards. For the no-salinity treatment (control), a 1 : 4 strength nutrient solution was used for all the flushing operations. One milliliter of broth containing approximately 10^8 rhizobia was added per seedling and this was repeated after 3 days to ensure a sufficient *Rhizobium* sp. population in the pot. The pots were flushed with the treatment solutions (0.25 l pot^{-1}) once every 4 days to avoid salt build-up. At the end of every day, the evapotranspirational water losses were made up by adding sterile deionized water. The pots were rerandomized every 4 days to minimize spatial effects in the greenhouse.

On the 65th day after sowing, the plants were harvested. The leaf area was measured with an automatic leaf-area meter (Delta T Devices Limited, England) and the nodulated roots were assayed for nitrogenase activity by acetylene reduction (Dart et al. 1972). After the acetylene reduction assay, the roots and nodules were cleaned of sand by washing in water and the nodules were separated and counted. The plant tops, roots, and nodules were dried at 70 °C for 48 h and the dry weights were recorded. Plant samples were finely ground by a Cyclone mill (UDY Corporation, Colorado, USA) for various chemical analyses. The N and P contents of the plant material were determined by a block digestion method and a Technicon autoanalyzer (Industrial Method No. 218–72 A). For the determination of Na, finely ground samples of 200–300 mg were digested with 6 ml tri-acid (HNO_3 : H_2SO_4 : $HClO_4$ at 10 : 0.5 : 2) in 50-ml volumetric flasks in a sand bath at 250 °C for 6–8 h (Piper 1952). The digested samples were diluted and analyzed by atomic absorption spectrophotometry (Varian, Model 1200). The chloride content in the plant samples was determined by Mohr's volumetric method (Blaedel and Meloche 1960).

Results and discussion

In all the *Rhizobium* sp. treatments, leaf area and shoot and root dry matter decreased with increasing salinity (Fig. 1). However, there were significant differ-

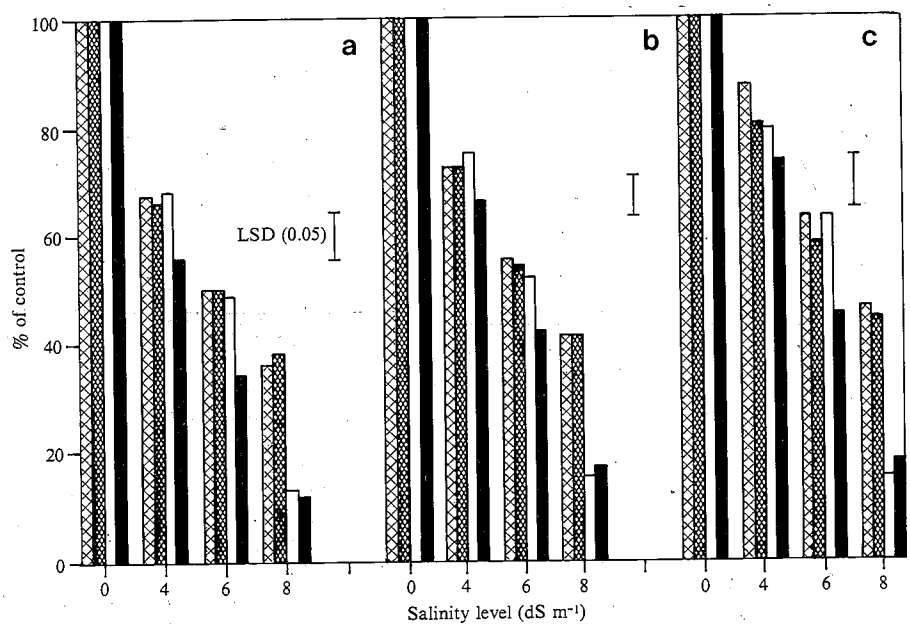

Fig. 1. Effect of salinity on **a** leaf area and **b** shoot and **c** root dry matter of pigeonpea genotype ICPL 227 inoculated with four *Rhizobium* strains. Data are means of four replications. 100% leaf area values 629, 614, 559, 586 ($cm^2\ pot^{-1}$); shoot dry matter values 4.84, 4.65, 4.32, 4.31, and root dry-matter values 1.22, 1.32, 1.25, and 1.23 g pot^{-1} for IC 3087 (☒), IC 3506 (▩), IC 3195 (□), and IC 3484 (■), respectively

ences in the salinity response among *Rhizobium* sp. treatments. *Rhizobium* sp. IC 3484 and IC 3195 were more affected by salinity than IC 3087 and IC 3506. This shows the variation among *Rhizobium* sp. strains in symbiotic ability with a common host under saline conditions.

For three of the *Rhizobium* sp. strains, the number of nodules decreased with increasing salinity, and at 8 dS m^{-1} there was more than 90% reduction in the plants inoculated with IC 3484 and IC3195 (Fig. 2). In contrast, the number of nodules in plants inoculated with IC 3087 increased with increasing salinity. These results confirm that *Rhizobium* sp. strains vary in their ability to nodulate under salinity stress. In several legumes, such as soybeans (Singleton and Bohlool 1984), pea (Siddiqui et al. 1985), cowpeas, mungbeans (Balasubramanian and Sinha 1976), and faba beans (Yousef and Sprent 1983), the total number of nodules was reported to decrease with increasing salinity. In soybeans, even 2.7 dS m^{-1} was able to suppress nodulation by 50% with nearly total suppression at 8.0 dS m^{-1} (Singleton and Bohlool 1984), a response similar to that observed with IC 3484. There appears to be no published work indicating an increase in the number of nodules with increasing salinity for any *Rhizobium* sp. strain, as occurred for IC 3087.

The total nodule dry matter decreased with increasing salinity in all *Rhizobium* sp. treatments (Fig. 2). However, the reduction with IC 3087 and IC 3506 was significantly less in comparison with IC 3484 and IC 3195. For IC 3506, IC 3195, and IC 3484, there was no significant change in the average nodule dry weight with increasing salinity (Table 2), and thus the decrease in the total nodule dry weight was merely a consequence of the reduced nodule number. This shows that nodule development is not affected by salinity, which is in agreement with the observations of Single-

Table 2. Effect of salinity on average nodule weight, specific nitrogenase activity, and leaf N and P of pigeonpea genotype ICPL 227 inoculated with different *Rhizobium* strains

Rhizobium strain	Salinity level (dS m^{-1})			
	0	4	6	8
Nodule weight (mg $nodule^{-1}$)				
IC 3087	0.57	0.33	0.31	0.28
IC 3506	3.12	2.89	3.60	3.16
IC 3195	1.88	1.74	1.82	2.20
IC 3484	2.78	2.53	3.65	2.07
LSD at 5% = 1.21				
Specific nitrogenase activity (μmol C_2H_4 g^{-1} dry $nodule$ h^{-1})				
IC 3087	91.7	105.5	86.4	97.6
IC 3506	115.6	84.8	90.7	114.6
IC 3195	101.3	71.8	83.6	192.9
IC 3484	109.3	66.5	97.1	114.6
LSD at 5% = 50.8				
Leaf N concentration (g kg^{-1} dry weight)				
IC 3087	31.9	32.0	32.3	33.9
IC 3506	32.0	32.5	33.0	34.9
IC 3195	33.1	33.2	32.8	25.5
IC 3484	33.0	29.5	29.3	29.2
LSD at 5% = 3.24				
Leaf P concentration (g kg^{-1} dry weight)				
IC 3087	1.5	1.5	1.7	2.2
IC 3506	1.6	1.6	1.6	2.4
IC 3195	1.5	1.7	1.9	2.8
IC 3484	1.6	1.5	1.7	2.4
LSD at 5% = 0.26				

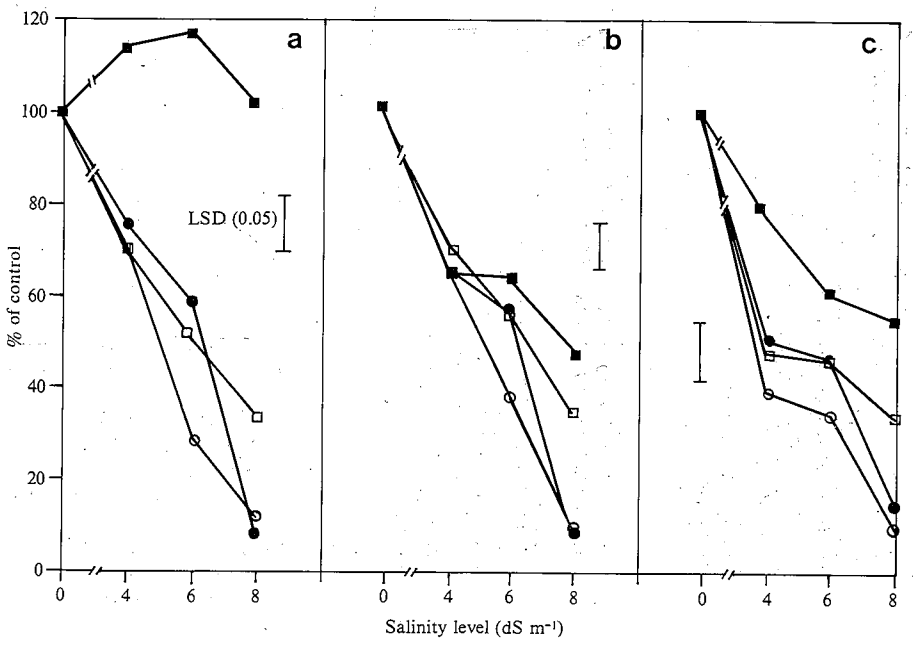

Fig. 2. Effect of salinity on **a** nodule number, **b** nodule dry matter, and **c** total nitrogenase activity of pigeonpea genotype ICPL 227 inoculated with four *Rhizobium* strains. Data are means of four replications. 100% nodule number 460, 135, 202, 153 (pot^{-1}); nodule dry weight 261, 400, 379, 419 (mg pot^{-1}); nitrogenase activity 24.0, 45.8, 38.3, and 45.0 (μmol C_2H_4 pot^{-1} h^{-1}) for IC 3087 (■), IC 3506 (□), IC 3195 (●), and IC 3484 (○), respectively

ton and Bohlool (1984). However, with IC 3087, the average nodule dry weight was reduced to 50% of the control (non-saline) at 8 dS m^{-1}, but this was not statistically significant due to the very low average nodule weight even in the control, in comparison with the other *Rhizobium* sp. strains. This was the main reason for the decrease in the total nodule dry weight with this *Rhizobium* sp. treatment, even though the total number of nodules increased with increasing salinity. It is not known whether the apparent reduction in the average nodule dry weight with IC 3087 was due to a direct salinity effect on nodule development or an indirect effect of the reduced shoot growth at 8 dS m^{-1}, causing a reduced supply of photosynthate to the symbiotic system.

The specific nitrogenase activity remained unaffected with increasing salinity in all the *Rhizobium* sp. treatments, except with IC 3195 at 8 dS m^{-1}, where there was a significant increase which could have been caused by a severe reduction in the total nodule dry weight (Table 2). This shows that nodule functioning in general remained unaffected with salinity stress in all of the *Rhizobium* sp. treatments. The decrease in total nitrogenase activity with increasing salinity (Fig. 2) would therefore be due to the reduction in total nodule dry matter.

There was no significant change in the leaf-N concentration with increasing salinity with IC 3087 and IC 3506, whereas with IC 3484 and IC 3195 leaf-N levels decreased with increasing salinity (Table 2); however the latter was significant only at 8 dS m^{-1}. This shows that *Rhizobium* sp. IC 3484 and IC 3195, which gave severe reductions in nodulation and N_2 fixation at 6 dS m^{-1} and above, were not able to maintain a N supply to the host under salinity stress, possibly leading to N deficiency. There was an increase in the leaf-P levels with increasing salinity (Table 2) in all the *Rhizobium* sp. treatments, suggesting that P uptake was not impaired by salinity. This is contrary to the findings of Wilson (1970) in soybeans, where P uptake was reported to be decreased with increasing salinity. Na and Cl levels in leaf, stem and roots of the pigeonpea increased with increasing salinity (Tables 3 and 4). There were no substantial differences among *Rhizobium* sp. treatments in this respect except at 8 dS m^{-1}, where shoot Na and Cl levels were significantly higher with IC 3195 and IC 3484 than with IC 3087 and IC 3506.

In previous studies, the selection of *Rhizobium* sp. strains for saline soils was made on the basis of the ability of strains to grow separately in saline yeast extract mannitol agar media (Yadav and Vyas 1971; Singleton et al. 1982). However, the salinity tolerance of free-living rhizobia is generally much higher than that of the host plant, and also, the tolerance of free-living *Rhizobium* sp. strains on yeast extract mannitol agar media does not correlate well with their symbiotic ability under saline conditions (Subbarao 1984; Table 1 and Fig. 1). The results of the present study have established that the variation in the growth of pigeonpea

Table 3. Effect of salinity on leaf, stem, and root Na concentrations in pigeonpea genotype ICPL 227 inoculated with different *Rhizobium* strains

Rhizobium strain	Salinity level (dS m^{-1})			
	0	4	6	8
Leaf Na concentration (g kg^{-1} dry weight)				
IC 3087	0.2	0.2	0.4	1.5
IC 3506	0.2	2.0	2.1	1.8
IC 3195	0.3	0.7	1.5	4.0
IC 3484	0.3	0.7	0.9	3.7
LSD = 0.94				
Stem Na concentration (g kg^{-1} dry weight)				
IC 3087	0.4	1.2	1.2	2.9
IC 3506	0.4	1.7	1.7	1.9
IC 3195	0.4	1.3	1.6	5.5
IC 3484	0.4	1.2	2.8	7.0
LSD = 0.95				
Root Na concentration (g kg^{-1} dry weight)				
IC 3087	5.6	11.3	15.0	16.0
IC 3506	5.6	12.0	12.5	16.0
IC 3195	6.0	13.1	15.5	18.5
IC 3484	6.5	12.5	17.0	18.5
LSD = 2.42				

Table 4. Effect of salinity on leaf, stem, and root Cl concentration of pigeonpea genotype ICPL 227 inoculated with different *Rhizobium* strains

Rhizobium strain	Salinity level (dS m^{-1})			
	0	4	6	8
Leaf Cl concentration (g kg^{-1} dry weight)				
IC 3087	0.7	12.7	26.2	40.5
IC 3506	0.7	13.1	26.2	36.4
IC 3195	0.7	12.6	27.0	44.4
IC 3484	0.7	13.0	32.3	44.0
LSD = 1.89				
Stem Cl concentration (g kg^{-1} dry weight)				
IC 3087	1.2	14.5	20.5	28.1
IC 3506	1.4	14.1	22.4	28.3
IC 3195	1.1	16.0	21.3	34.5
IC 3484	1.5	14.9	21.8	37.5
LSD = 2.36				
Root Cl concentration (g kg^{-1} dry weight)				
IC 3087	6.0	22.0	24.0	24.0
IC 3506	6.0	22.5	21.5	22.5
IC 3195	6.0	21.0	22.0	25.0
IC 3484	7.0	19.5	22.5	26.5
LSD = 2.84				

between the *Rhizobium* sp. treatments at different salinity levels was mainly due to the variation in their ability to form a symbiosis under saline conditions. This variation in symbiotic performance under saline conditions suggests that there is scope for the improvement of N_2 fixation of pigeonpea in saline soils through the selection of rhizobia based on their symbiotic performance. Although one of the most efficient *Rhizobium* sp. strains, IC 3087, was collected from a saline soil, the other equally efficient strain, IC 3506, was collected from a non-saline soil. This indicates that the *Rhizobium* sp. strains best able to form an effective symbiosis at high salinity levels are not necessarily derived from saline soils, as suggested by Bharadwaj (1975).

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References

Balasubramanian V, Sinha SK (1976) Effect of salt stress on growth, nodulation and nitrogen fixation in cowpea and mungbeans. Physiol Plant 36:197–200

Bernstein L, Ogata G (1966) Effects of salinity on nodulation and nitrogen fixation and growth of soybeans and alfalfa. Agron J 58:201–203

Bharadwaj KKR (1975) Survival and symbiotic characteristics of *Rhizobium* in saline-alkali soils. Plant and Soil 43:377–385

Blaedel WJ, Meloche VW (1960) Elementary quantitative analysis. Harper and Row, New York

Chauhan YS (1987) Screening for tolerance to salinity and waterlogging, case studies with pigeonpea and chickpea. In: Saxena NP, Johansen C (Sci-Editors) Adaptation of chickpea and pigeonpea to abiotic stresses. Proc Consultant's Workshop, ICRISAT, 19–21 Dec 1984, India, pp 93–103

Dart PJ, Day JM, Harris D (1972) Assay of nitrogenase activity by acetylene reduction. In: Use of isotopes for study of fertilizer utilization by legume crops. IAEA/FAO, Vienna, Technical Booklet, pp 85–100

Lakshmi-Kumari CS, Singh CS, Subbarao NS (1974) Root hair infection and nodulation in lucerne (*Medicago sativa*) as influenced by salinity and alkalinity. Plant and Soil 40:261–268

Lauter DJ, Munns DN, Clarkin KL (1981) Salt response of chickpea as influenced by N supply. Agron J 73:961–966

Piper CS (1952) Soil and plant analysis. Hans Publishers, Bombay

Siddiqui S, Kumar S, Sharma HR (1985) Studies on the effects of salinization on nodulation and nitrogen fixation in pea (*Pisum sativum*). Indian J Plant Physiol 28:369–375

Singleton PW, Bohlool BB (1984) Effect of salinity on nodule formation by soybean. Plant Physiol 74:72–76

Singleton PW, El-Swaify SA, Bohlool BB (1982) Effect of salinity on *Rhizobium* growth and survival. Appl Environ Microbiol 44:884–890

Subbarao GV (1984) Comparative salinity tolerance of pigeonpea, its rhizobial strains and symbiosis. M Tech Dissertation, Indian Institute of Technology, Kharagpur, India

Vincent JM (1970) A manual for the practical study of the root nodule bacteria. Blackwell, Oxford

Wilson JR (1970) Response to salinity in *Glycine*: VI. Some effects of a range of short term salt stress on the growth, nodulation and nitrogen fixation of *Glycine wightii*. Aust J Agric Res 21:571–582

Yadav NK, Vyas SR (1971) Response of root nodule rhizobia to saline-alkaline and acid conditions. Indian J Agric Sci 41:875–881

Yousef AN, Sprent JI (1983) Effects of NaCl on growth, nitrogen incorporation and chemical composition of inoculated and NH_4NO_3 fertilized *Vicia faba* L. plants. J Exp Bot 34:941–950

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