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Comparative salinity tolerance of symbiotically dependent and nitrogen-fed pigeonpea (*Cajanus cajan*) and its wild relative *Atylosia platycarpa**

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Summary. Once symbiosis between the pigeonpea cultivar ICPL 227 and the *Rhizobium* sp. strain IC 3024 is established, it is efficient in fixing N_2 under saline conditions and can support growth comparable to N-fed plants in growth media with up to 6 dS m^{-1} salinity. However, the early stages of establishment of the pigeonpea-IC 3024 symbiotic system have proved sensitive to salinity. The present study showed that the number of nodules was markedly reduced at 8 dS m^{-1} salinity; however, nodule development and functioning were not affected by salinity in the pigeonpea-IC 3024 symbiosis. The symbiotic system of *Atylosia platycarpa* and *Rhizobium* sp. strain IC 3087 was established successfully even at 12 dS m^{-1} and supported growth comparable to that of N-fed plants. P levels in leaves were increased under saline conditions in N-fed and N_2-fixing pigeonpea and *A. platycarpa*. There were no consistent differences in the leaf Na and chloride levels between N-fed and N_2-fixing plants of pigeonpea and *A. platycarpa*. The present study suggests that the rhizobial symbiosis may not be a necessary factor for initial screening of pigeonpea and related wild species for salinity tolerance.

Key words: *Atylosia platycarpa* – *Cajanus cajan* – Pigeonpea – N_2 fixation – *Rhizobium* – Salinity stress – Acetylene reduction assay

Salinity in arid and semi-arid regions constitutes a stress condition in crop plants that is of increasing importance in agriculture (Epstein 1978; Staples and Toenniessen 1984). Pigeonpea (*Cajanus cajan* [L.] Millsp.) is one of the important grain legumes cultivated in semi-arid regions where salinity problems can be acute (Chauhan 1987). Since the agricultural importance of legumes is closely related to their ability to fix N_2, any attempt to grow legumes under saline conditions needs to take into consideration the relative salt tolerance of the symbiotic N_2-fixation system (Läuchli 1984). Growth of soybean (Bernstein and Ogata 1966), *Glycine wightii* (Wilson 1970), and chickpea (Lauter et al. 1981) are more affected by salinity when grown symbiotically than when grown on mineral N. In alfalfa, however, the relative growth inhibition by salinity is similar to that for N-fed and N_2-fixing plants (Bernstein and Ogata 1966).

The present investigation was aimed at understanding the pigeonpea-*Rhizobium* sp. symbiotic system response to salinity stress and evaluating whether the symbiotic system was more sensitive to salinity than N-fed plants. An attempt was made to evaluate the salinity response of a well established symbiotic system compared with a symbiotic system established under salinity stress. The intention was to determine whether the initial stages of establishment of symbiosis were more sensitive to salinity than the functioning of an established symbiotic system. *A. platycarpa* has been identified as one of the wild relatives of pigeonpea that is most tolerant of salinity, among those wild relatives and cultivated pigeonpea genotypes that were tested (Subbarao 1988). Earlier studies identified *Rhizobium* sp. strain IC 3087 as the most symbiotically efficient under saline conditions (Subbarao 1988). The present investigation studied the response of the *A. platycarpa-Rhizobium* sp. (IC 3087) symbiosis to high levels of salinity and evaluated the relative salinity tolerance of *A. platycarpa* when dependent on either biologically fixed N_2 or mineral N.

Materials and methods

Rhizobium culture production

Rhizobium sp. strains used in this study were obtained from the pigeonpea-*Rhizobium* sp. culture collection of the Legumes Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India. They were all effective in fixing N_2 in symbiosis with pigeonpea. The cultures were maintained on yeast extract mannitol agar

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slopes (Vincent 1970). A loopful of the *Rhizobium* sp. culture was inoculated onto sterilized yeast extract mannitol broth and incubated on a rotary shaker at 28 °C for 3 days for IC 3024, a fast-growing *Rhizobium* sp., and 7 days for IC 3087, a slow-growing *Rhizobium* sp. After incubation, the culture was checked for purity by streaking a loopful of the broth culture on congo-red yeast extract mannitol agar.

Experiment 1

The growth medium consisted of sieved river sand which was washed and soaked for 24 h in acid solution (pH 1–2) and then thoroughly washed with tap water, dried and placed in 180-mm diameter polypropylene pots. The pots with sand were steam-sterilized. Seeds of pigeonpea (genotype ICPL 227) were surface-sterilized with 0.2% $HgCl_2$ solution for 5 min, washed in several changes of deionized water and then sown at the rate of eight per pot. The experiment had a randomized complete block design, replicated four times. The pots were placed in a greenhouse where temperatures were 28/22 °C (day/night) and relative humidity 60–70%.

The sand surface in each pot was covered with 50 g of polythene beads to minimize evaporational losses. The pots were watered with sterilized deionized water. 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* treatments: (1) N-fed (uninoculated); (2) IC 3024 early (inoculated with IC 3024 at the time of sowing); (3) IC 3024 late (inoculated with IC 3024 after the salinity treatments were imposed, that is 14 days after sowing); (e) IC 3506 late; (5) IC 3484 late; (6) IC 3087 late; and (7) IC 3195 late. The N-fed treatment was given 1.79 m*M* ammonium nitrate from 28 days after sowing. The results comparing treatments 4–6 have been given by Subbarao et al. (1990).

A modified Arnon and Hoagland N-free nutrient solution (0.25 strength) amended with NaCl+$CaCl_2$ (1:1 w/w) was used for the salinity treatments (NaCl and $CaCl_2$ concentrations were, respectively, 20 and 8 m*M* at 4 dS m^{-1}, 30 and 12 m*M* at 6 dS m^{-1}, and 40 and 16 m*M* at 8 dS m^{-1}). The composition of the nutrient solution (0.25 strength) in m*M* was: 0.23 KH_2PO_4, 0.52 KCl, 0.25 $MgSO_4$, 0.37 $CaCl_2$, 0.0015 $MnSO_4$, 0.00023 $ZnSO_4$, 0.00025 $CuSO_4$, 0.001 H_3BO_3, 0.00005 Na_2MoO_4, and 0.04 NaFeEDTA (ethylenediaminetetraacetic acid). The salinity treatments were imposed 14 days after sowing by flushing each pot with 1000 ml sterilized treatment solution. At the 0 salinity level, 0.25-strength nutrient solution (ECe 0.33 dS m^{-1}) was used for flushing. The *Rhizobium* sp. was inoculated by adding 1 ml of *Rhizobium* sp. broth (containing about 10^8 cells) per seed or seedling. This was repeated after 3 days to ensure sufficient a population of *Rhizobium* sp. in the pot. The pots were flushed with the respective treatment solutions (250 ml pot^{-1}) every 4 days to minimize salt build-up. At the end of each day, the evapotranspirational losses were adjusted by adding sterile deionized water to bring each pot to its designated soil water potential. The pot positions were rerandomized every 4 days to minimize spatial effects in the greenhouse. Plants were harvested 65 days after sowing.

At harvest, the leaf area was measured with an automatic leaf area meter (Delta T Devices Limited, England). Nodulated roots were assayed for nitrogenase activity by acetylene reduction (Dart et al. 1972). For this, the shoot was cut at the collar and the roots and nodules were carefully taken out and placed in a glass container with a rubber septum fitted in the lid. After a 30-min incubation in a 10% atmosphere of C_2H_2 at the ambient temperature, a 3.0-ml gas sample was removed and stored in a pre-evacuated venoject tube. The sample was analysed for C_2H_2 on a Pye Unicam 104 gas chromatograph fitted with a flame ionization detector and a 150-cm long glass column of 0.6 cm outside diameter, packed with Porapak N. The oven temperature of the gas chromatograph was 100 °C and the carrier gas (N_2) flow rate 45 ml min^{-1}. After the acetylene reduction assay, the roots and nodules were cleaned of sand by washing them in water and the nodules were separated and counted. The plant tops, roots, and nodules were dried at 70 °C for 48 h, and their dry weights recorded. Plant samples were finely ground by a cyclone mill (UDY Corporation, Colorado, USA) for chemical analysis. 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 of tri-acid (HNO_3:H_2SO_4:$HClO_4$ at 10:0.5:2) on a sand bath at 250 °C for 6–8 h (Piper 1952) in 50-ml volumetric flasks. The digested sample was 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).

Experiment 2

Seeds of *A. platycarpa* were scarified by nicking the testa with a scalpel, then surface-sterilized with 0.2% $HgCl_2$ solution for 5 min and washed with deionized water. Eight surface-sterilized seeds were sown in each pot. There were six salinity treatments, 0, 4, 6, 8, 10, and 12 dS m^{-1}, and five *N/Rhizobium* sp. treatments: (1) N-fed (uninoculated and 1.79 m*M* ammonium nitrate given from 28 days after sowing); (2) IC 3087 early (inoculated with *Rhizobium* sp. strain IC 3087 at the time of sowing); (3) IC 3087 late (inoculated with IC 3087 after salt treatment imposition, 14 days after sowing); (4) IC 3484 early and (5) IC 3484 late. The experimental design, growing conditions, and observations were similar to those in experiment 1. The plants were harvested 65 days after sowing. *Rhizobium* sp. strain IC 3484 was found to be non-infective for *A. platycarpa*. Hence the treatments involving IC 3484 were not included in the analysis of data.

Results and discussion

Leaf area and shoot and root dry matter decreased with increasing salinity with all the N treatments (N-fed, IC 3024 early, and IC 3024 late) of pigeonpea (Fig. 1, data presented for shoot dry matter only). The relative growth reduction with the IC 3024 early treatment was only slightly more than with the N-fed treatment. This indicates that the pigeonpea-IC 3024 symbiotic system, once established, could support growth comparable to that of N-fertilized plants under saline conditions. The IC 3024 late treatment suffered a much greater growth reduction than the other treatments at 6 and 8 dS m^{-1}, suggesting that establishment of the symbiotic system under a salinity stress is more adversely affected than the functioning

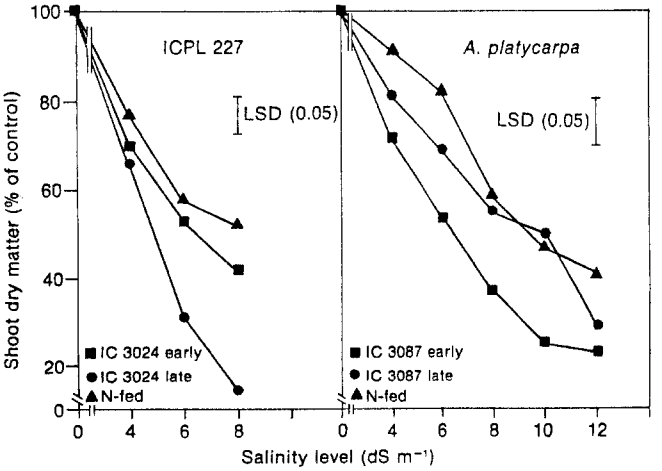

Fig. 1. Effect of salinity on shoot dry matter of N-fed and N_2-fixing pigeonpea (ICPL 227) and *Atylosia platycarpa*. The 100% shoot dry matter values (g pot^{-1}) for pigeonpea were 6.09, 4.84, and 6.0 for IC 3024 early (inoculation with *Rhizobium* at the time of sowing), IC 3024 late (inoculation 14 days after sowing), and N-fed treatments, respectively. For *A. platycarpa* the corresponding values were 7.03, 4.26, and 4.96 for IC 3087 early, IC 3087 late, and N-fed treatments, respectively

of an established system. In experiments with soybean (Bernstein and Ogata 1966), *Glycine wightii* (Wilson 1970), chickpea (Lauter et al. 1981), and *Vicia faba* (Yousef and Sprent 1983), the plants were more affected by salinity when grown symbiotically than under N fertilization, a response similar to that of the IC 3024 late treatment at 6 and 8 dS m^{-1} in the present study.

In *A. platycarpa,* the shoot dry matter decreased with increasing salinity similarly in the N-fed and IC 3087 late treatments (Fig. 1). With IC 3087 at 12 dS m^{-1}, the plants were pale green and showed N deficiency symptoms. Nevertheless, the symbiotic system of IC 3087-*A. platycarpa* was successfully established at up to 12 dS m^{-1} (IC 3087 late) and was efficient in fixing N_2 to support growth comparable to that of N-fed plants. However, the early inoculated treatment suffered a significantly higher reduction than the late inoculated and N-fed treatments. This is the reverse of the situation for ICPL 227 and the reasons are not clear.

The number of nodules per pot decreased with increasing salinity in the early- and late-inoculated IC 3024 treatments of pigeonpea (Table 1). The nodule number was reduced more with IC 3024 late than with IC 3024 early. Nodule initiation in the legume-*Rhizobium* symbiosis involves a complex interaction between host root hair, *Rhizobium* sp. strain, and the environment. Salinity may differentially affect each phase of the symbiosis: survival and growth in the rhizosphere of the host, *Rhizobium* infection of the host root hair, and nodule initiation and development. In general, *Rhizobium* strains can grow and survive at salt concentrations that are inhibitory to most agricultural legumes (Singleton et al. 1982). Our earlier studies on the salinity tolerance of pigeonpea rhizobia showed that many rhizobia could grow normally at 0.5% NaCl (8.5 dS m^{-1}) in yeast extract mannitol agar and that IC 3024 was one of the most tolerant among the tested strains (Subbarao 1984), because it could grow even at 7% NaCl (110 dS m^{-1}) in the yeast extract mannitol agar medium. Therefore, the survival and multiplication of IC 3024 in the rhizosphere of the host is not likely to be a limiting factor for establishing a successful symbiosis with pigeonpea at 6 and 8 dS m^{-1}. The severe reduction in the number of nodules with the late-inoculated IC 3024 treatment may therefore have been due to sensitivity in the root-hair infection process, or in the early stages of nodule formation, to the salinity stress. Similar views were expressed by Singleton and Bohlool (1984) for soybean and Lakshmikumari et al. (1974) for *Medicago sativa.*

Table 1. Effect of salinity on nodulation and N_2 fixation[a] by pigeonpea genotype ICPL 227 inoculated with *Rhizobium* strain IC 3024 at sowing (early) or 14 days after sowing (late)

Time of inoculation	Salinity level (dS m^{-1})				LSD[b] ($P = 0.05$)
	0	4	6	8	
Nodule number (pot^{-1})					
Early	176	120 (69)	106 (60)	77 (45)	
Late	183	95 (52)	36 (20)	6 (3)	21.9
Total nodule dry weight (mg pot^{-1})					
Early	307	200 (65)	177 (58)	149 (49)	
Late	348	221 (64)	175 (50)	33 (9)	42.0
Average nodule weight (mg $nodule^{-1}$)					
Early	1.76	1.67	1.68	1.95	
Late	1.90	2.33	4.99	5.48	1.21
Total nitrogenase activity (μmol C_2H_4 $pot^{-1}h^{-1}$)					
Early	52.3	21.5 (42)	19.3 (37)	18.5 (36)	
Late	53.3	20.3 (39)	16.0 (30)	4.8 (9)	5.18
Specific nitrogenase activity (μmol C_2H_4 g^{-1} dry nodule h^{-1})					
Early	171	111	110	127	
Late	156	92	92	174	50.8

[a] Absolute values, with percentage of control in parentheses
[b] Least significant difference

In *A. platycarpa*, in symbiosis with the IC 3087 *Rhizobium* sp. strain, the number of nodules increased with salinity level up to 10 dS m^{-1}, and then decreased at 12 dS m^{-1}, with the late-inoculated treatment (Table 2). However, with the IC 3087 early treatment, the number of nodules decreased at 8 dS m^{-1} and higher salinity levels. Thus the IC 3087–*A. platycarpa* symbiotic system became established and was able to function even at 12 dS m^{-1}, a salinity level which is toxic to many crop legumes. In many legumes, including soybean (Singleton and Bohlool 1984), pea (Siddiqui et al. 1985), cowpea and mungbean (Balasubramanian and Sinha 1976), and *Vicia faba* (Yousef and Sprent 1983), nodulation was reported to be severely affected by salinity. In *Glycine max*, even 2.7 dS m^{-1} was able to suppress nodulation to 50%, with nearly total suppression at 8.0 dS m^{-1} (Singleton and Bohlool 1984), a response similar to the response of the pigeonpea-IC 3024 symbiotic system in the present study.

There was a reduction in the total nodule dry mass in IC 3024 early and late treatments with increasing salinity (Table 1). This was mainly due to reduced nodule numbers since there was no significant change in the average nodule dry mass in the early, and a very large increase in the late, IC 3024 treatments. This increase in the individual nodule mass in the IC 3024 late treatment to some extent compensated for the greater reduction in the number of nodules in this treatment under the higher salinity levels.

In *A. platycarpa,* the total nodule dry mass decreased at 8 dS m^{-1} and higher salinity levels, even though the number of nodules was increased at up to 10 dS m^{-1} and remained unaffected at 12 dS m^{-1} in the IC 3087 late treatment. This was mainly due to a reduction in the average nodule dry mass, indicating that nodule development was affected by salinity, in contrast to the pigeonpea-IC 3024 symbiotic system. This may have been due to a reduced photosynthate supply from the shoot to the symbiotic system, since shoot growth was affected by salinity. Even in the N-fed plants, shoot growth was reduced to about 50% at 12 dS m^{-1}. In the pigeonpea-IC 3024 symbiotic system, the severe reduction in the number of nodules at 8 dS m^{-1} (about 97% reduction), is probably the main reason for the maintenance or increase in individual nodule size.

Total nitrogenase activity decreased under saline conditions in the early and late IC 3024 treatments of

Table 2. Effect of salinity on nodulation and N_2 fixation[a] of *Atylosia platycarpa* inoculated with *Rhizobium* IC 3087 at sowing (early) or 14 days after sowing (late)

Time of inoculation	Salinity level (dS m^{-1})						LSD[b] ($P = 0.05$)
	0	4	6	8	10	12	
Nodule number (pot^{-1})							
Early	124	131 (107)	116 (94)	88 (72)	81 (66)	89 (72)	
Late	131	157 (120)	164 (126)	162 (124)	183 (139)	118 (90)	18.3
Total nodule dry weight (mg pot^{-1})							
Early	167	124 (76)	93 (58)	72 (44)	40 (25)	40 (25)	
Late	105	96 (93)	100 (97)	81 (80)	60 (58)	26 (24)	22.4
Average nodule dry weight (mg nodule^{-1})							
Early	1.36	0.94	0.81	0.83	0.49	0.45	
Late	0.80	0.61	0.61	0.50	0.33	0.22	0.213
Total nitrogenase activity (μmol C_2H_4 pot^{-1}h^{-1})							
Early	5.17	3.65 (72)	2.11 (41)	2.15 (42)	1.03 (20)	0.24 (5)	
Late	5.29	3.85 (73)	2.38 (45)	2.27 (43)	1.05 (20)	1.05 (20)	0.51
Specific nitrogenase activity (μmol C_2H_4 g^{-1} dry nodule h^{-1})							
Early	32.2	30.3	23.1	31.4	26.8	6.3	
Late	51.6	40.4	24.3	28.4	17.9	49.2	16.9

[a, b] See footnotes to Table 1

Table 3. Effect of salinity on leaf N, P, Na, and chloride concentrations in pigeonpea genotype ICPL 227 dependent on symbiotically fixed N or combined N

N source	Salinity level (dS m^{-1})				LSD[a] ($P = 0.05$)
	0	4	6	8	
Leaf N concentration (g kg^{-1} dry weight)					
IC 3024 early	31.5	31.4	30.5	32.8	
IC 3024 late	32.5	28.5	24.4	24.5	
N-fed	32.0	32.0	36.2	40.1	3.24
Leaf P concentration (g kg^{-1} dry weight)					
IC 3024 early	1.5	1.6	1.6	2.6	
IC 3024 late	1.5	1.6	1.8	2.6	
N-fed	1.5	1.5	1.8	2.6	0.26
Leaf Na concentration (g kg^{-1} dry weight)					
IC 3024 early	0.3	1.5	1.9	3.0	
IC 3024 late	0.2	1.1	1.7	4.3	
N-fed	0.7	1.2	1.7	2.5	0.94
Leaf chloride concentration (g kg^{-1} dry weight)					
IC 3024 early	0.5	13.8	25.9	39.2	
IC 3024 late	0.5	12.1	31.8	48.4	
N-fed	0.6	14.1	24.3	39.2	1.89

[a] Least significant difference

pigeonpea (Table 1). This might have been merely a consequence of the reduction in the total nodule dry mass since there was no major change in the specific nitrogenase activity under saline conditions. Thus, in pigeonpea, nodule functioning was little affected by salinity levels, a response similar to that reported in soybean (Singleton and Bohlool 1984), *Macroptilium atropurpureum*, and *Glycine wightii* Wilson (1985). In *A. platycarpa* also, the total nitrogenase activity decreased under saline conditions with the early and late IC 3087 treatments (Table 2). However, the total nitrogenase activity of *A. platycarpa* even in the control (non-saline) plants was very low, possibly because the plants were close to maturity. The specific nitrogenase activity of the early-inoculated treatment was unaffected by salinity levels of up to 10 dS m^{-1} but declined at 12 dS m^{-1} (Table 2). In the late-inoculated treatment, this parameter was significantly reduced at 6–10 dS m^{-1}, but not at 12 dS m^{-1} (Table 2).

Leaf-N levels of pigeonpea increased at 6 dS m^{-1} salinity and above with the N-fed treatment, but decreased with the late IC 3024 and did not change with the early IC 3024 treatment (Table 3). This suggests that with the late IC 3024 treatment, where nodulation was severely affected at 6 and 8 dS m^{-1}, the symbiotic system was not able to meet the N requirements of the host, leading to N deficiency. In *A. platycarpa*, with IC 3087 early, there was no significant change in the leaf-N levels while with IC 3087 late, this parameter decreased under increasing salinity (Table 4). The pod-N levels in both the early and the late IC 3087 treatments were increased with increasing salinity (Table 4). The N levels in stem and root were unaffected by salinity (data not presented). With the N-fed treatment of *A. platycarpa*, the N levels in leaf, pod, stem, and root increased with increasing salinity (Table 4, data presented only for leaf and pod). The reduction in leaf-N levels under saline conditions in the IC 3087 late treatment may not be a limiting factor for growth in *A. platycarpa*, at least up to 10 dS m^{-1}, because there were no N-deficiency symptoms and no clear differences in the relative reduction in growth between the N-fed and the IC 3087 late treatments.

Leaf-P levels in N-fed and IC 3024 treatments of pigeonpea increased with increasing salinity (Table 3). In *A. platycarpa* also, there was an increase in the leaf-P levels in the N-fed, IC 3087 early, and IC 3087 late treatments under saline conditions; however, in the IC 3087 late treatment, it was not significant (Table 4). This is

Table 4. Effect of salinity on leaf N, P, Na, and chloride concentrations in *Rhizobium* (IC 3087)-inoculated and N-fed *Atylosia platycarpa*

N source	Salinity level (dS m^{-1})						LSD[a] ($P = 0.05$)
	0	4	6	8	10	12	
Leaf N concentration (g kg^{-1} dry weight)							
IC 3087 early	29.3	27.9	25.5	28.4	23.9	24.8	
IC 3087 late	36.1	33.1	32.5	29.6	27.5	25.4	
N-fed	22.8	31.0	35.4	45.2	46.2	45.9	7.87
Pod N concentration (g kg^{-1} dry weight)							
IC 3087 early	23.4	25.1	25.2	27.1	29.6	27.8	
IC 3087 late	26.1	27.1	28.1	28.6	28.0	32.9	
N-fed	20.8	22.4	23.0	30.6	34.9	35.4	4.34
Leaf P concentration (g kg^{-1} dry weight)							
IC 3087 early	1.4	1.5	1.7	1.8	2.1	2.3	
IC 3087 late	1.8	1.6	1.6	1.9	1.8	2.0	
N-fed	1.6	1.8	2.2	2.4	2.5	2.7	0.47
Leaf Na concentration (g kg^{-1} dry weight)							
IC 3087 early	0.7	0.7	0.7	0.5	1.0	2.3	
IC 3087 late	0.5	0.5	0.5	0.7	0.6	1.3	
N-fed	0.5	0.5	0.6	0.7	0.6	0.8	0.35
Leaf chloride concentration (g kg^{-1} dry weight)							
IC 3087 early	1.5	27.5	36.4	41.6	48.1	51.6	
IC 3087 late	1.2	17.5	27.0	36.0	33.4	42.1	
N-fed	1.2	25.6	40.9	46.0	46.1	46.2	5.5

[a] Least significant difference

contrary to the findings of Wilson (1970) in soybean, where P levels decreased under saline conditions in plants inoculated with *Rhizobium* sp. The present study with pigeonpea and its wild relative *A. platycarpa* suggests that the availability of P in N-fed and N_2-fixing plants may not be a growth-limiting factor under saline conditions.

Na and chloride levels in leaf tissue increased with increasing salinity in the N-fed and IC 3024 treatments of pigeonpea (Table 3). There were no clear trends between the N-fed and the IC 3024 treatments in leaf, stem, and root Na, and chloride levels under saline conditions (data presented only for leaf Na and chloride). However, at 8 dS m^{-1}, the leaf Na and chloride levels of the IC 3024 late treatment were significantly higher than those in the N-fed and IC 3024 early treatments. In *A. platycarpa*, there were no clear trends between the N-fed and N_2-fixing plants in their leaf Na and chloride levels (Table 4), indicating that the ion-uptake behavior of either cultivated pigeonpea or *A. platycarpa* is not much influenced by the type of N acquisition under saline conditions. This is not in agreement with the observations made by Yousef and Sprent (1983) in *Vicia faba*, who reported a higher uptake of Na and chloride in *Rhizobium*-inoculated plants than in N-fed plants.

The present results demonstrate that the pigeonpea-*Rhizobium* sp. (IC 3024) symbiotic system, once established, is efficient in fixing N_2 under saline conditions and that N may not be a growth-limiting factor. However, the early stages of symbiotic establishment appear to be sensitive to salinity, particularly at 6 and 8 dS m^{-1}, while nodule function remains little affected. However, the response of the *A. platycarpa*-IC 3087 symbiotic system, where the number of nodules was enhanced or unaffected by salinity levels, even up to 12 dS m^{-1}, a salinity level that is toxic to many crop legumes, indicates that symbiotic sensitivity to salinity stress varies between symbioses. However, with early inoculation for the IC3087-*A. platycarpa* symbiosis, the response was different and we cannot explain this with present knowledge. There are several reasons to suggest that it is appropriate to use N-fed systems to evaluate the salinity tolerance of germplasm of pigeonpea and its wild relatives. First, the symbiotic function seems less sensitive than the plant itself to saline conditions (Fig. 1), although the infection process in cultivated pigeonpea is more sensitive and may require a separate screening procedure. Second, the interactions between *Rhizobium* and pigeonpea in the salinity response (Subbarao et al. 1990) could complicate the detection of plant genotypic differences, in which case differences would be specific to a particular *Rhizobium* strain. Third, the uptake of sodium and chloride to shoots, which largely accounts for genotypic differences in the salinity response among pigeonpea and its wild relatives (Subbarao 1988), is not greatly modified by the mode of N acquisition.

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