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Effects of Salinity on N_2 Fixation, Nitrogen Metabolism and Export and Diffusive Conductance of Cowpea Root Nodules

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Abstract

N_2 fixation and nodule functioning were assessed following application of 40 or 80 mol m^{-3} NaCl to root systems of an established cowpea symbiosis (21–22 day, *Vigna unguiculata* L. Walp cv. Vita 3: *Bradyrhizobium* strain CB 756). Liquid culture techniques were used which precluded the initiation and development of new nodules and prevented direct exposure of the surface of nodules to salt during the period of study. Both levels of NaCl inhibited photosynthetic CO_2 fixation (75 and 88%), decreased stomatal conductance, increased substomatal CO_2 level and decreased the total water potential of leaves. Despite these severely negative effects of NaCl on photosynthetic parameters of the plant, the respiration of nodulated roots increased for up to 12 d following NaCl treatment and, although nitrogenase activity was depressed by NaCl, substantial rates of acetylene reduction, $^{15}N_2$ fixation and N export from nodules were maintained. Data are interpreted to indicate that the continued high "sink strength" of the root system in attracting substrates to maintain an enhanced level of respiration also serves to maintain a supply of oxidizable substrates to the nodules. NaCl treatment caused significant changes in the relative concentrations of nitrogenous solutes of xylem; ureides were reduced and asparagine was increased.

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However, increased asparagine was not due to changes in the spectrum of exported products of N_2 fixation, but was rather a result of N mobilisation in the plant. Nodules from salt-treated plants showed evidence of lowered conductivity to O_2 suggesting that, in addition to maintenance of a supply of assimilates, the symbiosis also adjusted to the effects of salt by increasing the resistance of a variable gaseous diffusion barrier.

Keywords: cowpea, diffusive conductance, legume, N_2 fixation, nodules, *Rhizobium*, salinity, symbiosis, *Vigna unguiculata*, *ureides*

1. Introduction

Increases in the salinity of soils or or water supplied used for irrigation result in decreased productivity of most crop species. The physiological features of "salt-stress" and of the responses of plants in adapting to and surviving under saline conditions have been extensively studied (Flowers et al., 1977; Greenway and Munns, 1980; Hansen and Hitz, 1982), and, although only a limited number of legume species have been assessed, most have been found to be relatively sensitive to salt (Downton, 1984).

Salt-stress leads to inhibition of photosynthesis, both as a consequence of reduced leaf diffusive conductance and also due to direct ionic effects on the intermediary metabolism of CO_2 fixation (Greenway and Munns, 1980; Seemann and Critchley, 1985; Plaut et al., 1990). In legumes N_2 fixation is closely dependent on photosynthesis through the supply of carbon substrates translocated to nodules and it is therefore likely that nodule metabolism will be limited as a consequence. Yousef and Sprent (1983) found that N accumulation by *Vicia faba* plants dependent on N_2-fixation was more sensitive to NaCl than was N accumulation by plants supplied with $NH_4 NO_3$, suggesting that nodule functioning may indeed be more susceptible to the effects of salt stress than other plant organs. Saline conditions also interfere with symbioses by reducing infection of root hairs by *Rhizobium* (Zahran and Sprent, 1986) and by inhibiting nodulation (Yousef and Sprent, 1983; Singleton and Bohlool, 1984; Craig et al., 1991). *Rhizobium* species generally grow at considerably higher salt levels than their host legumes (Singleton et al., 1982; Bhardwaj, 1975; Craig et al., 1991) so that the degree of tolerance of the host determines the success of compatible bacterial strains in forming an effective symbiosis (Craig et al., 1991). Singleton and Bohlool (1983) used a well established soybean symbiosis cultured in a split root system to distinguish between direct and indirect effects of salt on N_2 fixation. They concluded that the functional processes of nodules were not directly affected by salt, but rather lowered leaf water potentials and arrested leaf expansion caused inhibition of nitrogenase activity.

A central feature of symbiotic functioning of legume nodules is the operation of a variable diffusion barrier, possibly in the inner cortical region of the organ (Layzell and Hunt, 1990), which serves to adjust the flux of O_2 entering infected tissues in response to the level of respiratory activity of bacteroids. In this way variations in O_2 consumption, occasioned as a result of reduced phloem delivery of sugars, of exposure of nodules to combined N, to drought stress or to inhibitors of pathways of N metabolism, do not result in excessive O_2 accumulation and toxicity (reviewed in Layzell and Hunt, 1990).

In view of the demonstrated response of diffusional adjustment to drought and water deficit (Weisz et al., 1985; Durand et al., 1987) it was of interest to see if similar adjustments in nodule functioning accompanied salt-stress. Cowpea (*Vigna unguiculata* (L.) Walp) was chosen as a species which has shown clear evidence of gaseous diffusional adjustment of nodules (Dakora and Atkins, 1990a,b) and in which photosynthesis, leaf conductance and growth are inhibited by low to moderate levels of NaCl salinity (Plaut et al., 1990). The present study examines the response of nodulated root systems of cowpea in liquid culture to different levels of NaCl applied after an effective symbiosis had been established and the plants were totally dependent on high rates of N_2 fixation for their N nutrition.

2. Materials and Methods

Plant material

Seeds of cowpea (*Vigna unguiculata* (L.) Walp, *cv* Vita 3) were inoculated with a commercially-prepared peat suspension of *Bradyrhizobium* strain CB 756 (Nitrogerm, Root Nodule Pty Ltd., Woy Woy NSW Australia), germinated and cultured in washed sand with a nutrient solution free of combined N in a naturally lit glass house in Perth, Western Australia with 28–32° C day and 15–20° C night temperatures (Atkins et al., 1984). After 7 d, seedlings were transferred to liquid culture and grown in nutrient solution-free of combined N as described previously (Atkins et al., 1984). Twenty-one to twenty-two days thereafter, the nutrient solution was modified to contain 0, 40 or 80 mol m^{-3} NaCl. This solution was replaced twice weekly and deionised water added on a daily basis so as to maintain the level of liquid just below the zone of nodulation.

Throughout the duration of experiments, nodulation was confined to the crown and principally on the tap root. Thus, effects due to salt on N_2 fixation were restricted to the activities of the initial crop of nodules, the cultural

conditions preventing subsequent nodulation (Atkins et al., 1984). Furthermore, because the level of nutrient solution was maintained below the zone of nodulation, the outer surface of nodules was not directly exposed to NaCl.

All measurements of gas exchange by leaves and nodulated roots were made on plants maintained under the same cultural conditions.

Collection and analysis of solutes in xylem exudate and nodule extracts

At periods from 27 to 42 d after planting (i.e. 6 to 21 d after addition of NaCl) the shoots of 5 plants were removed and xylem exudate collected as bleeding sap from the root stumps (Atkins et al., 1990). The rates of exudation were recorded (Pate et al., 1979). Samples were stored at −20° C prior to analysis for their contents of ureides, amino acids and amides using methods described previously (Trijbels and Vogels, 1966; Atkins et al., 1988). Total amino acid N was measured colorimetrically with ninhydrin (Yemm and Cocking, 1955).

Nodules were collected from root systems and water soluble compounds recovered from ethanol extracts as described earlier (Atkins et al., 1980b). Their contents of ureides, amino acids and amides were measured as above and organic acids and sugars separated and measured using an HPLC technique (Pate et al., 1985).

Recovery and analysis of products of $^{15}N_2$ fixation

Liquid cultures containing 5 plants exposed to 80 mol m^{-3} NaCl in the nutrient solution for 7 d were sealed and 10% (v/v) $^{15}N_2$ (96 atom % excess ^{15}N) supplied in the head space of the container for 4 hr. The 5 plants in a container were removed, separated into roots, nodules, stems and leaves and plunged into liquid N_2 for storage prior to extraction. Samples of the tissues were freeze-dired, finely ground and their $^{15}N/^{14}N$ ratio determined by mass spectrometric analysis following Kjeldahl digestion and recovery of ammonia (Atkins et al., 1988). In addition, samples of nodules were extracted and the ^{15}N content of amino acids, amides and ureides determined by mass spectrometry following their chromatographic separation and recovery as described previously (Atkins et al., 1988).

Nitrogenase assay

Nitrogenase (EC 1.7.99.2) was measured by acetylene reduction in a continuous flow-through assay system similar to that described by Minchin et al. (1983). The enclosed root systems of intact plants (5 per culture vessel), grown for 27 d in the absence of salt or exposed to 40 or 80 mol m^{-3} NaCl for

1–12 d prior to assay, were transferred to a flowing stream of air for 30 min before acetylene (10%; v/v) was added to the stream. In some experiments the pO_2 of the gas was also adjusted at this time to 10, 20, 30, 40 or 50% O_2 (v/v; with a balance of N_2) and one ml samples taken at 5 min intervals up to 35 min for analysis of acetylene and ethylene content by gas liquid chromatography (Atkins et al., 1984). The gases were mixed and supplied to the enclosed nodulated root system as described by Dakora and Atkins (1990a).

Measurement of CO_2 by nodulated root systems

Production of CO_2 by enclosed nodulated root systems was measured continuously as the change in CO_2 concentration of a flowing gas stream (air) passing through liquid culture vessels each with 5 plants. The gas from the vessels was passed to a gas exchange circuit (Pate and Atkins, 1983) which incorporated a differential infra red gas analyzer (ADC Model 2130 Analytical Development Co., Ltd., Hoddesdon, Herts UK) and an automated programmable switching device which allowed the sequential sampling and assay of the effluent from 6 vessels, one of which contained nutrient solution, but no plants, and served as a control.

Measurement of CO_2 exchange, stomatal conductance and water potential of leaves

Rates of net CO_2 exchange (APS, apparent photosynthesis) and substomatal CO_2 concentrations at saturating light flux were calculated from measurements of pCO_2 entering and leaving a cuvette enclosing a known area of leaf and attached to a portable infra-red open gas analysis system (ADC LCA system; Analytical Development Co., Ld, Hoddesdon, Herts UK). Stomatal conductance to water vapour was calculated from measurements of H_2O exchange made with an automated diffusion porometer (Delta-T Devices, UK). Total water potential of leaves was measured on cut shoots using a pressure chamber (Richie and Hinckley, 1975). All measurements were made using the first- and second-formed trifoliolate leaves at three times during the photoperiod of clear, sunny days.

3. Results

Addition of 40 or 80 mol m^{-3} NaCl to the nutrient solution bathing nodulated roots of cowpea resulted in marked inhibition (75 and 88% respectively) of net CO_2 uptake by leaves, decreased stomatal conductance, significant increases in the substomatal concentration of CO_2 and progressively more negative total

Table 1. Effect of NaCl, added to the nutrient solution surrounding nodulated roots of cowpea, on CO_2 exchange stomatal conductance and total water potential of leaves

Measurement*	Control	40 mM NaCl	80 mM NaCl
		μmol CO_2 $m^{-2} \cdot s^{-1}$	
APS	13.28±1.50**	3.25±1.90	1.56±1.50
Stomatal conductance		mol $m^{-2}s^{-1}$	
abaxial	0.36±0.09	0.23±0.04	0.06±0.01
adaxial	0.77±0.47	0.63±0.04	0.19±0.10
		Pa	
Substomatal p(CO_2)	23.3±1.7	29.5±1.9	33.7±2.9
Leaf total		MPa	
Water potential	−0.11±0.02	−0.32±0.10	−0.47±0.15

* Measurements were made on the 1st and 2nd trifoliolate leaves 7–14 d after the application of NaCl. Data for each parameter at each time were very similar and were combined.

** Values are means ±SE (n = 12–15 for APS, n = 12–18 for stomatal conductance, n = 8–10 for sub-stomatal CO_2 and n = 6–8 for leaf water potential).

Table 2. Effect of NaCl, applied to the nodulated root system of cowpea plants for 7 d, on the solute compostion of nodules

Solute	Control	80 mol m^{-3} NaCl
	(μmol g^{-1} FW nodules)	
Succinate	6.3±0.5*	1.9±0.2
Malate	0.3±0.1	0.2±0.1
Total organic acids**	6.9±0.6	2.3±0.3
Sucrose	19.2±2.5	46.7±8.3
Glucose	11.8±3.4	28.0±3.5
Fructose	4.0±0.2	3.3±1.0
Asparagine	1.28±0.52	0.89±0.16
Glutamine	0.18±0.03	0.07±0.01
Aspartate	0.47±0.08	0.87±0.15
Glutamate	1.87±0.49	2.12±0.36
Other amino acids***	4.20±0.86	2.78±0.21
Total amino acids	8.00±1.98	6.76±0.99

* Mean ±SE (n = 3)

** Included succinate, malate, citrate, tartrate, malonate, oxalate and fumarate

*** Included threonine, serine, glycine, alanine, valine, methionine, isoleucine, leucine, γ amino butyrate, histidine, ammonia, lysine and arginine

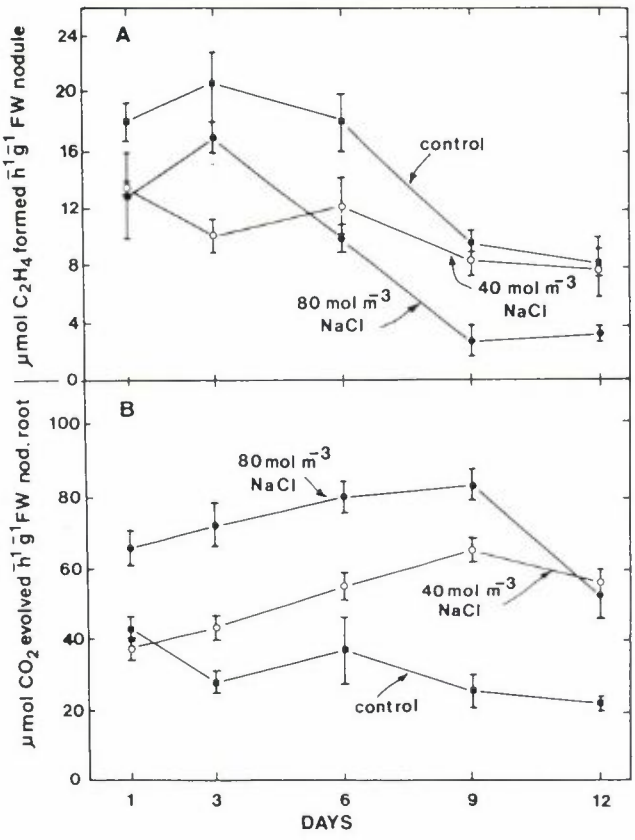

Figure 1. Nitrogenase activity (A) and respiratory CO_2 evolution (B) by nodulated root systems of intact cowpea plants exposed to 0, 40 or 80 mol m^{-3} NaCl for periods from 1 to 12 d. Values are means and bars indicate ±SE (n = 3 pots each of 5 plants).

leaf water potentials (Table 1). Despite the severely reduced photosynthetic CO_2 fixation and, presumably, supply of newly fixed sugars in phloem, rates of respiration, up to twice those of controls, were maintained by the nodulated root system for up to 12 d following addition of salt (Fig. 1B). Nitrogenase activity was also inhibited by salt (Fig. 1A). However, with both levels of NaCl, relatively high rates of acetylene reduction (67% and 56% of controls at 40 and 80 mol m^{-3} respectively) were maintained up to 6 d after treatment. At later times the acetylene reducing activity of the controls also declined and were not significantly different to plants exposed to 40 mol m^{-3} salt after 12 d (Fig. 1A). During this period, the dry weight of nodules on plants was unchanged.

Application of NaCl altered the composition of solutes in nodules (Table 2).

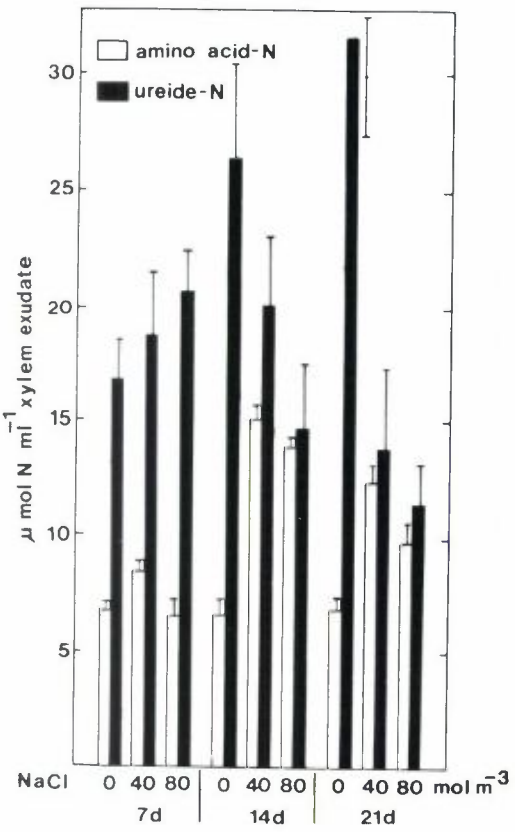

Figure 2. The contents of ureide-N and amino acid-N in xylem exudate collected from nodulated cowpea plants exposed to 0, 40 or 80 mol m^{-3} NaCl for 7, 14 or 21 d. Values are means and bars indicate ±SE n = 3–5).

Total organic acids were reduced by more than two-thirds, largely as a result of significantly lower concentrations of succinate, while sucrose and glucose levels more than doubled, increasing the overall solute content of the nodules of salt-treated plants by almost 44 μmol g^{-1} fresh weight of tissue. The overall concentration of amino acids and amides in nodules fell slightly following salt treatment. This was due to relatively modest changes in a wide range of compounds rather than a substantial alteration of one or two amino acids (Table 2).

Salt treatment reduced the rate at which xylem exudate was formed at the

Table 3. Effect of NaCl, applied to the nodulated root system of cowpea for 14 d, on the composition of amino compounds in xylem exudate

Compound	Control	80 mol m^{-3} NaCl
	nmol ml^{-1}	
Aspartate	295.6±42	756.3±37
Threonine	127.4±21	251.0±33
Serine	158.5±12	282.7±13
Asparagine	2862.6±165	9209.6±400
Glutamate	61.1±8	369.4±12
Glutamine	321.1±25	908.2±35
Glycine	7.0±1	27.7±4
Alanine	65.5±7	281.7±20
Valine	166.7±12	265.7±13
Methionine	11.9±3	13.3±4
Isoleucine	60.6±4	46.9±6
Leucine	53.0±2	96.9±4
Tyrosine	14.5±3	70.0±5
Phenylalanine	22.4±4	43.9±11
γ amino butyrate	5.8±2	348.5±1
Histidine	95.5±4	299.7±23
Ammonium	116.3±20	271.8±24
Lysine	267.7±19	187.4±57
Arginine	117.8±29	188.4±11
Total	6832.1	13919.6

Mean ±SE (n = 2–3)

cut root surface of plants from which the shoots had been excised. Control plants exuded around 50 μl sap h^{-1} while those treated with 40 or 80 mol m^{-3} NaCl for 7 d exuded 26 and 12 μl h^{-1} respectively. Longer periods of exposure to salt reduced exudation further; so that by 21 d values of 4–6 μl h^{-1} were recorded. After 7 d exposure to 40 or 80 mol m^{-3} NaCl, the relative composition of nitrogenous solutes in xylem exudate was similar to that of controls (Fig. 2); the ureides (allantion + allantoic acid) accounting for 70–75% of the N. However, after 14 or 21 d ureide-N in xylem was significantly reduced by the salt treatments, accounting for little more than 50% of xylem-borne N. In contrast, there was a significant increase in the levels of amino acid-N in xylem exudate of plants after 14 or 21 d exposure to salt. More detailed analysis of the individual amino acids in xylem inducated that, while the levels of many had increased due to salt treatment, the change in asparagine

Table 4. Distribution of ^{15}N between plant organs and between solutes of nitrogen in nodules of cowpea plants following exposure of the nodulated root system to 10% (v/v) $^{15}N_2$ (96 atom% xs) for 4 hr. Plants exposed to salinity were cultured in nutrient solution containing 80 mol m^{-3} NaCl for 7 days before being used.

(A) Organ	Control		80 mol m^{-3} NaCl	
Nodules	288.50	(44)*	87.80	(45)
Roots	9.90	(2)	7.90	(4)
Stems	178.00	(27)	30.40	(15)
Leaves	179.20	(27)	70.20	(36)
Total	655.60		196.30	
(B) Solute				
Aspartate	2.85	(3)	1.56	(6)
Asparagine	0.80	(1)	0.11	(1)
Glutamate	9.40	(10)	2.50	(9)
Glutamine	2.20	(2)	0.98	(4)
Serine	2.87	(3)	0.67	(2)
Ureides	75.00	(81)	21.25	(79)
Total	93.12		27.07	

* Values in parentheses are % of total ^{15}N recovered

Table 5. Effect of NaCl, added to the nutrient solution surrounding the nodulated root system of cowpea plants for 7 d, on maximum rates of acetylene reduction assayed in a range of atmospheres containing different pO_2

pO_2	Control	80 mol m^{-3} NaCl
10	8.4*	2.4
20	9.8	3.7
30	6.7	4.9
40	4.2	4.1
50	3.9	2.3

* Rates are maximum values found by assaying C_2H_4 in the flowing gas stream leaving pots containing 5 plants each every 5 min up to 35 min. The weight of nodules was 94 ± 12 mg dry weight $plant^{-1}$ for controls and 90 ± 4 mg dry weight $plant^{-1}$ for those receiving 80 mol m^{-3} NaCl.

level was most significant (Table 3). Thus, of the 7.09 μmol ml^{-1} increase in total amino acids of xylem, the increase in asparagine alone (6.35 μmol mol^{-1}) accounted for close to 90%. While it is not possible to directly equate exudation rate from decapitated plants with the rate of xylem transport in the intact plant, changes in exudation coupled with those in N solute composition of exudate due to salt indicate a significant reduction in the amount of N transferred from nodules to the shoot.

Exposing the nodulated root systems of plants treated with 80 mol m^{-3} NaCl for 7 d to $^{15}N_2$ showed that, although N_2 fixation was inhibited (in this particular case by close to 70%), nodules were still able to export products of current fixation to the rest of the plant (Table 4A). In fact, the proportional distribution of ^{15}N to organs other than nodules was much the same in salt-treated plants as it was in the controls. A more detailed analysis of ^{15}N labeling of nitrogenous solutes within nodules indicated that the proportional distribution of label was not markedly altered by salt (Table 4B); in both cases 80% of the ^{15}N was recovered as ureide-N with glutamate (10%) being the next most heavily labeled compound.

The degree to which nodules on salt-treated plants had altered their gaseous conductivity was assessed by measuring rates of ethylene efflux (from acetylene reduction) at a range of pO_2. In plants which received no NaCl the highest rates of nitrogenase activity were found at 20% O_2, while in those supplied 80 mol m^{-3}NaCl for 7 d, higher rates were found at 30 or 40% than at 20% O_2 (Table 5).

4. Discussion

The symbiosis was established and fully developed prior to the application of NaCl. Furthermore, the liquid culture conditions used precluded the initiation of new nodules so that effects of NaCl on processes of infection, nodule initiation and differentiation were avoided. Thus only the effects of salinity on nodule functioning were considered, and, in view of the fact that direct contact between nodules and nutrient solution was prevented, these were limited to effects of NaCl on processes outside the nodule or to consequences of translocated salt.

The effects of salt, added to the nutrient solution around roots, in inhibiting photosynthetic CO_2 fixation and decreasing the stomatal conductance of leaves are similar to those described previously for the species by Plaut et al. (1990). Although the present study has not evaluated the relative contributions of stomatal and non-stomatal factors in limiting photosynthesis under

saline conditions, increased sub-stomatal CO_2 concentration is consistent with effects of NaCl on both transpiration and metabolic processes of CO_2 fixation.

One probable consequence of severely reduced CO_2 fixation by leaves would have been a marked reduction in the loading of currently synthesised sugars to phloem and their translocation to sink organs, particularly to the nodulated root system. Despite this, NaCl treatment stimulated respiration of nodulated roots, with increased rates of CO_2 evolution being maintained for at least 12 d. In the case of plants exposed to 80 mol m^{-3} NaCl, where CO_2 release by the root system was doubled compared to untreated plants, it seems reasonable to suppose that this elevated level of "salt respiration" (Beevers, 1961), possibly reflecting elevated rates of active ion transport, was supported largely by oxidizable substrates derived from stored resources of the shoot or of the root system itself. Similarly, nitrogenase activity, although reduced following salt addition to plants, was maintained at significant rates for at least 12 d. Furthermore, nodules continued to export fixed N to the roots and shoot organs of salt-treated plants even though their transpirational activities were reduced.

There is some precedent for the idea that when current photosynthetic products are in short supply, substrates can be mobilised from reserves to sustain N_2 fixation. An earlier study of diurnal variation in the functioning of cowpea nodules (Rainbird et al., 1983) showed that in the dark period, carbohydrate supplies were maintained, in part by hydrolysis of non structural carbohydrate within the nodule, and in part by continued import of translocated sugars from the shoot. As a result, high levels of N_2 fixation were maintained in the absence of leaf photosynthesis, and, even though the transpirational activity of the shoot was reduced by more than 90%, some export of the products of fixation to all organs of the plant was maintained in darkness. Nieman et al. (1988) have suggested that the maintenance of "sink activity" is an important property of roots in their adaptation to salt and it does not seem unreasonable to suppose that this activity serves also to maintain a supply of oxidizable substrates to nodules and N_2 fixation.

The apparent tolerance of nodules to salt found in these experiments contrasts with the results of Sprent (1972) and Ikeda et al. (1992) who showed that nitrogenase activity was inhibited very severely and rapidly (e.g. after 5 min exposure to KCl or NCl; Sprent, 1972). However, in these earlier studies, salt solution was applied directly to the surface of nodules which were detached, thus largely precluding the chances of osmotic regulation. In the present study, nodules did not come into direct contact with nutrient solution so that any salt entering the organ would have done so through processes of translocation or from radial diffusion within the root. Singleton and Bohlool (1983) also found that the functioning of nodules was relatively tolerant of salt supplied to either

the nodulated or non-nodulated halves of a split root system in soybean. They concluded that the inhibition which did occur (50%) resulted from effects of salt on leaf water potential and expansion rather than from direct effects on nitrogenase activities.

In view of the disruption of water relations in the plant following exposure to salt and the likelihood that similar effects might also have occurred in nodules, it is of interest to compare the results of this study with those of studies into effects of water deprivation on nodule functioning. In soybean, droughting results in progressive inhibition of nitrogenase activity (Huang et al., 1975a,b) together with a concomitant and progressive decrease in gaseous conductance of nodules (Weisz et al., 1985), both parameters reflecting nodule water potential (Durand et al., 1987). However, inhibition of photosynthesis was negligible (5%) by comparison with inhibition of nitrogenase (70%) and Durand et al. (1987) suggested that water stress exerts effects on nitrogenase activity which are independent of the rate of photosynthesis and involves increases in the resistance of nodules to diffusion of O_2. Irigoyen et al. (1992) also noted that nitrogenase activity of alfalfa nodules was more susceptible to reduced water potential than was the rate of photosynthetic CO_2 exchange of leaves. These authors (Irigoyen et al., 1992) suggest that reduction of photosynthate supply is not the primary reason for drought-stress induced inhibition of nodule functioning, but rather, that water deficit directly affects metabolic processes in nodules. The response of nodules to salt in the present study was clearly quite different to that of drought and indicates that, either there was little change in nodule water potential as a result of applying salt to the root system alone, or that continued assimilate supply allowed effective osmoregulation and N_2 fixation.

Recently Irigoyen et al. (1992) have shown that water stress leads to an accumulation of proline and total soluble sugars by nodules of alfalfa. They suggest that these solutes may serve to aid the maintenance of turgidity under conditions of lowered water potential. Cowpea nodules showed no evidence of proline accumulation with salt stress but did accumulate a substantially higher level of soluble sugars, particularly sucrose. These might serve to achieve some osmotic adjustment within the tissue. Similar increases in sucrose, and to a lesser extent glucose and proline, have been described for alfalfa nodules by Fougère et al. (1991) who also showed increases in D-pinitol and asparagine which could indicate that a range of solutes serve osmoregulatory functions in different symbioses under saline conditions.

The data showing stimulation of maximum rates of acetylene reduction by supra ambient pO_2 in salt-treated plants are consistent with their nodules having reduced gaseous conductivity compared to those of controls. A similar

response to a range of different treatments has been noted for cowpea and for other symbioses (Dakora and Atkins, 1990b; Layzell and Hunt, 1990). Although the mechanisms which underlie the control of gaseous fluxes in nodules have not been defined, it is perhaps not surprising that salt treatment, like droughting and consequent water stress, causes such a response. Layzell and Hunt (1990) concluded that any treatment which reduces the phloem supply of carbohydrates to nodules is likely to result in their optimization of O_2 flux by diffusional adjustment. Thus, although nodules in these studies maintained some "sink strength" in attracting translocated solutes, the supply was less than controls and as a consequence O_2 diffusion was limited to a greater degree in response to salt treatment.

Exposure to NaCl has been shown to reduce the pools of nucleotides in tissues of susceptible species (Nieman et al., 1988) and, in root tips especially, normal metabolism is altered so that the oxidation of purine bases is accelerated relative to *de novo* purine synthesis or purine salvage (Peterson et al., 1988). Thus, the relatively high levels of ureides in xylem of salt-treated plants could reflect increased purine breakdown in roots or other tissues rather than their continued export as products of N_2 fixation from nodules. The significant level of ^{15}N recovered in xylem-borne ureides following $^{15}N_2$ supply does not entirely preclude additional production from nucleic acid catabolism but it indicates that a large proportion of the ureide molecules in xylem were indeed derived from fixation. On the other hand, it seems likely that enhanced levels of amino compounds in xylem, recorded after prolonged exposure to salt, resulted from changes in N metabolism which were independent of N_2 fixation. $^{15}N_2$ supply showed that salt did not alter the spectrum of N-solutes exported from nodules and that the high level of asparagine in xylem of salt-treated plants was not the product of current fixation. Assimilation of inorganic N by roots in cowpea leads to the synthesis and translocation of asparagine (Atkins et al., 1980a) and it seems reasonable to suppose that under saline conditions the elevated xylem-borne levels of this amide reflect a high rate of reassimilation of ammonia, released as a result of increased protein and amino acid catabolism in the plant as a whole.

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