MIRCEN Journal, 1987, 3, 235–241

Effect of ammonium nitrate on nodulation and nitrogen fixation (acetylene reduction) of the tropical legume *Sesbania rostrata*

A. Moudiongui & G. Rinaudo*

Laboratoire de Microbiologie des Sols, ORSTOM, B.P. 1386, Dakar, Senegal

Received 18 July 1986; revised and accepted 16 March 1987

Introduction

The effect of combined nitrogen on the physiology of the *Rhizobium* legume symbiosis has been the subject of many studies (reviewed by Gibson 1976; Munns 1977; Rigaud 1981). Addition of mineral nitrogen in soil or synthetic medium affects both nodulation and nitrogen fixation. The degree of effects observed depends on the concentration and the form of nitrogen compounds, the time of application, the cultivation conditions, and finally the type of plant and bacteria used.

Sesbania rostrata is a tropical legume which displays the unusual feature of both stem and root nodulation when associated symbiotically with the specific rhizobia ORS 571 (Dreyfus & Dommergues 1981) or WE 7 (Olsson *et al.* 1984). When the difference method, the balance method or the isotope-dilution method are used, nitrogen fixation by *S. rostrata* is about 200 kg N fixed/ha in 50 days (Rinaudo *et al.* 1983; Rinaudo & Moudiongui 1986). *Sesbania rostrata* shows a typical root-hair infection in which a meristematic zone is initiated prior to nodulation (Olsson & Rolfe 1985). Stem nodulation occurs by an alternative infection procedure referred to as 'crack entry' in which a pre-existing meristematic zone is taken over for nodule development (Duhoux 1984; Olsson & Rolfe 1985).

In a previous study (Dreyfus & Dommergues 1980), *S. rostrata* was grown hydroponically in the presence of 3 mmol/l NH_4NO_3. Root nodulation was inhibited, while stem nodulation and related nitrogen fixation (acetylene reduction) were not affected. Similar effects were then observed with other stem-nodulated legumes: *Aeschynomene scabra* (Eaglesham & Szalay 1983) and *A. afraspera* (Becker *et al.* 1986).

The present study was initiated to determine the effect of various concentrations of NH_4NO_3 on both stem and root nodulation and nitrogen fixation (acetylene reduction activity) of *S. rostrata*.

*Département F, ORSTOM, 213 rue LaFayette, 75480 Paris Cedex 10, France, and to whom correspondence should be addressed.

Materials and methods

Glasshouse procedure

The experiment was done under natural daylight in a glasshouse with a temperature of 32°C by day and 25°C by night.

Sesbania rostrata seeds were treated with conc. H_2SO_4 for 30 min, then washed thoroughly with sterile water and germinated on moist filter paper in Petri dishes. Seedlings were transferred to 15-cm plastic pots containing N-free autoclaved silica sand and gravel (one seedling per pot). The basic nutrient solution consisted of the following (mg/l): $CaSO_4 \cdot 2H_2O$, 138; K_2CO_3; 55.2; $K_3PO_4 \cdot 2H_2O$, 99; $MgSO_4 \cdot 7H_2O$, 49.3; NaCl, 23.4; Fe (as NaFe-EDTA); 4; Gibson trace elements, 1 ml/l (Vincent 1970); pH 6.8. For one month before beginning experimental treatments, the plants were watered on alternate days with 50 ml sterile solution of the above nutrients containing in addition 1 mM NH_4NO_3.

Sesbania rostrata was inoculated 30 days after sowing: a 2-day-old ORS 571 *Rhizobium* culture (10^9 cells/ml) was sprayed along the stems. The soil was inoculated with the same culture to induce root nodulation. Symbiosis-related parameters were evaluated 20 days after inoculation.

Automatic irrigation system

After inoculation, the plants were continuously irrigated with an automatic system. Four concentrations of combined nitrogen were used: 0, 1.5, 3.0 and 6.0 mM NH_4NO_3. For each treatment, a 25-litre nutrient medium reservoir was connected to a multichannel peristaltic pump which irrigated simultaneously five free-draining pots. The daily rate of application of the nutrient medium was about 300 ml per pot, to maintain the initial nitrogen concentration during the whole experiment.

Acetylene reduction activity

Twenty days after inoculation (50 days after planting), the plants were harvested, and roots and stems were assayed individually for acetylene reduction activity (ARA) (Hardy *et al.* 1968). Nodules were removed and counted. Then shoots, roots and nodules were dried (70°C) and weighed. Total nitrogen analysis was performed with an automated Kjeldahl system (Büchi 322).

Results

Increasing NH_4NO_3 levels resulted in positive responses in plant height, dry weight and nitrogen content (Table 1).

Table 2 shows the effects of different concentrations of NH_4NO_3 on symbiotic parameters. Control plants and plants grown at 1.5 and 3.0 mM NH_4NO_3, had approximatively the same stem nodule number. The stem nodule size was strongly affected at 6.0 mM NH_4NO_3 as shown in Fig. 1. The root nodule number of plants grown at 1.5 mM NH_4NO_3 was about 50% of the root nodule number of control plants. Root nodules were not observed in plants grown at 3.0 mM NH_4NO_3 or more.

The stem nodule activity decreased by 65% at 1.5 mM, 85% at 3.0 mM and was completely inhibited at 6.0 mM. Root nodule activity was more affected: it decreased by 81% at 1.5 mM and was completely inhibited at 3.0 mM or more (Table 2).

Table 1 Effect of NH_4NO_3 concentration on plant height, plant dry weight and nitrogen content

NH_4NO_3 applied (mM)	Plant height (cm)	Plant dry weight (g/plant)	Plant nitrogen content (mg/plant)
0	990 a	6.45 a	192.0 a
1.5	1058 a	7.45 ab	242.0 b
3.0	1226 b	8.31 bc	291.0 c
6.0	1270 b	9.38 c	420.0 d

Numbers followed by the same letter in a column are not significantly different at 5% level by Duncan's multiple range test (5 replicates).

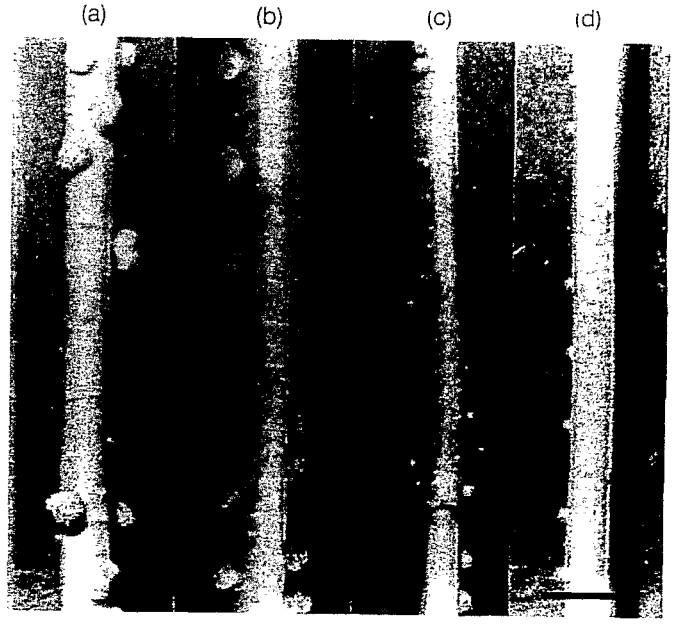

Fig. 1 Stem nodulation of *S. rostrata* grown with various concentrations of NH_4NO_3, 20 days after inoculation with *Rhizobium* ORS 571. A, Control without N supplied: B, 1.5 mM NH_4NO_3; C, 3.0 mM NH_4NO_3; D, 6.0 mM NH_4NO_3. Scale bar indicates 1 cm.

Discussion

Nodulation and related nitrogen fixation (acetylene reduction activity) of the tropical legume *S. rostrata* are more affected on the roots than on the stems. The difference in response of root and stem nodules to combined nitrogen can be attributed to a number of factors.

Localised effect of combined nitrogen

An effect of this type has been noted for split root systems. Thus in the experiment conducted by Carrol & Gresshoff (1983) using white clover, both sides of the split root

Table 2 Effect of NH_4NO_3 concentration on acetylene reduction activity (ARA), nodule mass, and nodule number

NH_4NO_3 applied (mM)	Nodule dry weight (g/plant)		Nodule number		ARA (μmol C_2H_4 produced/plant/h)		Specific activity*	
	S	R	S	R	S	R	S	R
0	0.10 a	0.11 a	50.50 a	156.0 a	7.72 a	6.35 a	77.2 a	58.0 a
1.5	0.08 b	0.07 b	51.60 a	84.2 b	2.76 b	1.15 b	36.8 b	17.0 b
3.0	0.06 b	–	43.40 a	–	1.15 c	–	19.0 c	–
6.0	–	–	–	–	–	–	–	–

*Specific activity is expressed as μmoles of ethylene formed per gram dry weight of nodules per hour.
Notes: S, stem; R, roots. Numbers followed by the same letter in a column are not significantly different at the 5% level by Duncan's multiple range test (5 replicates).

system were inoculated with *Rhizobium*, but only one side received nitrate: inhibition of nodulation was localised, occurring only on roots in direct contact with nitrate, but nitrogenase activity of nodules formed on the nitrate-free root portion was affected. We obtained similar results: in the presence of 3 mM NH_4NO_3, the effect of combined nitrogen resulted in root nodulation inhibition; the formation of stem nodules was not significantly affected, but their activity (acetylene reduction) decreased by 85% (Table 2).

In contrast, Dreyfus & Dommergues (1981) found that stem nodulation and nitrogen fixation of *S. rostrata* grown in test tubes, were not only unaffected, but even enhanced by the presence of 3 mM NH_4NO_3. The low frequency of renewal of the nutrient medium might explain the non-inhibition observed in their experiment.

Carbohydrate deprivation

The effects of combined nitrogen on symbiotic nitrogen fixation have frequently been attributed to deprivation of photosynthetically-derived materials in the nodules owing to prior use of carbohydrate in nitrate assimilation and growth (Oghoghorie & Pate 1971; Gibson & Pagan 1977; Houward 1980; Wong 1980). Such an hypothesis might explain the alleviation of inhibition of stem nodule activity by NH_4NO_3, the carbohydrate availability being greater in stem nodules than in root nodules.

Infection processes

The difference in the ability of stems and roots of *S. rostrata* to nodulate in the presence of NH_4NO_3, may be partially explained by the differences between the two types of infection processes involved. Root infection occurs via root hairs, which are very sensitive to combined nitrogen: curling of root hairs and infection thread formation are reduced by nitrate applications (Munns 1968); and nitrate prevents lectin synthesis thereby blocking the adsorption of rhizobia onto root hairs (Dazzo & Brill 1978). In contrast, the stem infection process, in which root hairs are not involved, appears to be less sensitive to mineral nitrogen.

Species of *Sesbania* and *Aeschynomene* have in common the ability to tolerate water-logged conditions. Thus stem nodulation appears as a probable adaptive response to water-logging. This ability confers on these legumes an advantage for assimilating both soil and atmospheric nitrogen.

References

Becker, M., Alazard, D. & Ottow, J. C. G. 1986 Mineral nitrogen effect on nodulation and nitrogen fixation of the stem-nodulating legume *Aeschynomene afraspera*. *Zeitschrift für Pflanzernährung und Bodenkde* **149**, 485–491.

Carrol, B. J. & Gresshoff, P. M. 1983 Nitrate inhibition of nodulation and nitrogen fixation in white clover. *Zeitschrift für Pflanzenphysiologie* **110**, 77–88.

Dazzo, F. B. & Brill, W. J. 1978 Regulation by fixed nitrogen of host symbiont recognition in the *Rhizobium* clover symbiosis. *Plant Physiology* **62**, 18–21.

Dreyfus, B. & Dommergues, Y. R. 1980 Non inhibition de la fixation d'azote atmosphérique par l'azote combiné chez une légumineuse à nodules caulinaires *Sesbania rostrata*. *Comptes Rendus de l'Académie des Sciences, Paris D* **291**, 767–770.

Dreyfus, B. & Dommergues, Y. 1981 Nitrogen fixing nodules induced by *Rhizobium* on the stems of the tropical legume *Sesbania rostrata*. *FEMS Microbiology Letters* **10**, 313–317.

DUHOUX, E. 1984 Ontogénèse des nodules caulinaires de *Sesbania rostrata* (légumineuse). *Canadian Journal of Botany* **62**, 982–994.
EAGLESHAM, A. R. J. & SZALAY, A. A. 1983 Aerial stem nodules on *Aeschynomene* spp. *Plant Science Letters* **29**, 265–272.
GIBSON, A. H. 1976 Recovery and compensation by nodulated legumes to environmental stress. In *Symbiotic Nitrogen Fixation in Plants*, IBP 7, ed. Nutman, P. S. pp. 385–403. London: Cambridge University Press.
GIBSON, A.H. & PAGAN, J.D. 1977 Nitrate effects on the nodulation of legumes inoculated with nitrate-reductase deficient mutants of *Rhizobium*. *Planta* **134**, 17–22.
HARDY, R.W.F., HOLSTEN, R.D., JACKSON, E.K. & BURNS, R.C. 1968 The acetylene ethylene assay for N_2-fixation: laboratory and field evaluation. *Plant Physiology* **43**, 1185–1207.
HOUWARD, F. 1980 Influence of ammonium and nitrate nitrogen on nitrogenase activity of pea plants as affected by light intensity and sugar addition. *Plant and Soil* **54**, 271–282.
MUNNS, D.N. 1968 Nodulation of *Medicago sativa* in solution cultures. III. Effect of nitrate on root hairs and infection. *Plant and Soil* **29**, 33–47.
MUNNS, D.N. 1977 Mineral nutrition and legume symbiosis. In *A Treatise on Dinitrogen Fixation, Section IV, Agronomy & Ecology*, ed. Hardy, R.W.F. & Gibson, A.H. pp. 353–391. New York: John Wiley & Sons Inc.
OGHOGHORIE, C.G.O. & PATE, J.S. 1971 The nitrate stress syndrome of the nodulated pea (*Pisum arvense* L.). Techniques for measurement and evaluation in physiological terms. *Plant and Soil*, Special Volume, ed. Lie, T.A. & Mulder, E.G. pp. 185–202. The Hague. The Netherlands: Martinus Nijhoff.
OLSSON, J.E., ROLFE, B.G., SHINE, J., PLANZINZKI, J., NAYUDU, M., RIDGE, R. & DART, P. 1984 The biological characterization of *Sesbania rostrata* infection by *Rhizobium* spp. In *Advances in Nitrogen Fixation Research*, 715, ed. Veeger, C. & Newton, W.E. The Hague, The Netherlands: Martinus Nijhoff.
OLSSON, J.E. & ROLFE, B.G. 1985 Stem and root nodulation of the tropical legume *Sesbania rostrata*, by *Rhizobium* strains ORS 571 and WE 7. *Journal of Plant Physiology* **121**, 199–210.
RIGAUD, J. 1981 Comparison of the efficiency of nitrate and nitrogen fixation in crop field. In *Nitrogen and Carbon Metabolism*, ed. Bewley, J.D. pp. 17–48. The Hague: Martinus Nijhoff.
RINAUDO, G., DREYFUS, B. & DOMMERGUES, Y. R. 1983 *Sesbania rostrata* green manure and the nitrogen content of rice crop and soil. *Soil Biology and Biochemistry* **15**, 111–113.
RINAUDO, G. & MOUDIONGUI, A. 1986 Fixation d'azote par *Sesbania rostrata*: son utilisation comme engrais vert. *Bulletin des Recherches Agronomiques de Gembloux* (in press).
VINCENT, J.M. 1970 *A Manual for the Practical study of Root-Nodule Bacteria*, IBP Handbook No. 15. Oxford: Blackwell Scientific Publications.
WONG, P.P.1980 Nitrate and carbohydrate effects on nodulation and nitrogen fixation (acetylene reduction) activity of lentil (*Lens esculenta* Moench). *Plant Physiology* **66**, 78–81.

Summary

The effect of ammonium nitrate on nodulation and nitrogen fixation (acetylene reduction activity) was investigated using *Sesbania rostrata*, a tropical legume with the ability to nodulate both roots and stems. Plants were grown in plastic pots on silica sand and gravel, inoculated one month after sowing and then continuously irrigated with an automatic irrigation system. Four nitrogen treatments were applied: 0, 1.5, 3.0 and 6.0 mM NH_4NO_3. Related symbiotic parameters were evaluated 20 days later. With 3.0 mM NH_4NO_3, root nodulation was inhibited. At that concentration, stem nodulation was not affected, but related nitrogenase activity decreased 85% and was completely inhibited at 6.0 mM NH_4NO_3. Increasing NH_4NO_3 concentration resulted in a diminution of stem nodule size. Stem and root acetylene reduction were strongly inhibited by high mineral nitrogen concentrations; however root nitrogen fixation was more affected than stem nitrogen fixation.

Résumé

L'effet du nitrate d'ammonium sur la nodulation et la fixation d'azote (réduction de l'acétylène) a été étudié avec la légumineuse tropicale *Sesbania rostrata*, qui présente la particularité de noduler à la fois sur la tige et sur les racines. Les plantes ont été cultivées dans des pots en plastique sur un mélange constitué de sable siliceux et de gravier. Un mois après le semis, elles ont été inoculées et placées sous irrigation continue au moyen d'un système automatique permettant l'apport du milieu nutritif. Quatre concentrations en azote ont été utilisées: 0, 1,5, 3,0 et 6,0 mmol/l NH_4NO_3. Les paramètres symbiotiques ont été analysés 20 jours plus tard. La nodulation racinaire est totalement inhibée en présence de 3,0 mmol/l NH_4NO_3. Cette concentration en azote combiné n'a pas d'effet apparent sur la formation des nodules de tige, mais leur activité nitrogénase diminue de 85%; leur activité est totalement inhibée en présence de 6,0 mmol/l NH_4NO_3. L'augmentation de la concentration en nitrate d'ammonium, se traduit par une diminution de la taille des nodules de tige. Les fortes concentrations en azote combiné ont un effet inhibiteur très marqué sur les activités réductrices d'acétylène racinaire ou caulinaire de *S. rostrata*; toutefois cet effet est plus sensible au niveau racinaire qu'au niveau caulinaire.

Resumen

Efecto del nitrato amónico en la nodulación y la fijación de nitrógeno (reducción de acetileno) de la leguminosa tropical Sesbania rostrata

Se estudió el efecto del nitrato amónico en la nodulación y la fijación de nitrógeno, medida mediante la reducción de acetileno, utilizando *Sesbania rostrata*, una leguminosa tropical que forma nódulos tanto en raíz como en tallo. Las plantas se cultivaron en arena de sílice y grava, en macetas de plástico, se inocularon un mes después de la siembra y a partir de este momento se regaron continua y automáticamente. Se aplicaron cuatro tratamientos de N; 0, 1.5, 3.0, y 6.0 mM NH_4NO_3 Los parámetros relativos a la simbiosis se evaluaron 20 días después. La nodulación en raíz se inhibió con 3.0 mM NH_4NO_3, sin embargo a esta concentración la nodulación en tallo no estaba inhibida aunque la actividad nitrogenasa disminuyó en un 85%, quedando totalmente inhibida a 6 mM NH_4NO_3. El incremento en la concentración de nitrato amónico produjo una disminución del tamaño de los nódulos del tallo. Elevadas concentraciones de nitrógeno mineral inhibieron fuertemente la reducción de acetileno tanto a nivel de raíz como de tallo, aunque la fijación de nitrógeno resultó más afectada en la raíz.

MIRCEN Journal, 1987, 3, 235–241

Effect of ammonium nitrate on nodulation and nitrogen fixation (acetylene reduction) of the tropical legume *Sesbania rostrata*

A. Moudiongui & G. Rinaudo*

Laboratoire de Microbiologie des Sols, ORSTOM, B.P. 1386, Dakar, Senegal

Received 18 July 1986; revised and accepted 16 March 1987