

EFFECT OF NITROGEN ON NODULATION AND SYMBIOTIC NITROGEN FIXATION IN COWPEA CULTIVAR MI 35—A LABORATORY STUDY

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ABSTRACT

The effect of four concentrations 0, 0.5, 1.0 and 1.5 mM of potassium nitrate (KNO_3) on nodulation and N_2 fixation in cowpea cultivar MI—35 inoculated with rhizobium strains CIAT 301 and SLM 602 was investigated in a Leonard jar experiment under growth cabinet conditions ($25^\circ/22^\circ\text{C}$ day/night temperature, 14 hour photoperiod with photosynthetic photon flux density of $500 \mu\text{E}/\text{sq. m}/\text{sec}$). The N_2 fixation (nodule activity) expressed as acetylene reduction rate in μM ethylene per gram nodule fresh weight per hour, reached the maximum at 1.0 mM KNO_3 in plants inoculated with the rhizobium strain CIAT 301 and decreased at 2.0 mM KNO_3 . In plants inoculated with rhizobium strain SLM 602 the nodule activity remained about the same at 1.0 and 2.0 mM KNO_3 . The activity was lowest at 0.5 mM KNO_3 in both rhizobium treatments. The nodule fresh weight increased with increasing nitrogen level up to 1.0 mM KNO_3 in both treatments inoculated with rhizobium strains SLM 602 and CIAT 301. There was no further increase in nodule fresh weight beyond 1.0 mM KNO_3 concentration. However the plant dry weight continued to increase with increasing N concentration in a similar manner in both rhizobium treatments. Nitrogen level affected nodule activity and plant dry weight in the cowpea cultivar MI—35. Significant benefits could be obtained by the proper combination of rhizobium inoculum and N level.

KEY WORDS : Cowpea (*Vigna unguiculata*), Nitrogen, Nitrogen fixation, Nodulation

INTRODUCTION

The effect of combined nitrogen on nodulation and symbiotic N_2 fixation is not clearly understood. When nitrogen is available in the root medium (soil or substrate) the leguminous plants even when nodulated, uses it preferentially. The nodules remain almost inactive but will be ready to function once the soil nitrogen has been exhausted. Nodules may remain small and inactive during the entire growing season when there is a large reservoir of nitrogen available in the

soil. High concentration of combined nitrogen generally has been shown to inhibit rhizobial infection and nodule development (Pankhurst and Jones, 1979). Effects of low levels of combined nitrogen have been reported to be either inhibitory or stimulatory on N_2 fixation and nodulation (Dart and Mercer, 1965). It has also been reported that combined nitrogen in the root medium generally delays or inhibits nodulation (FAO, 1984).

Cowpea cultivar MI—35, popularly known as “Lanka Parippu” has been one of the important grain cowpea varieties grown in the dry regions of Sri Lanka. Rhizobium capable of nodulating cowpea belongs to a poorly defined group which consists of “Cowpea miscellany” and nodulates a variety of legumes, tropical and sub-tropical in origin (Broomfield and Rao, 1983). Specific information available on the symbiotic relationship of cowpea cultivar MI—35 and rhizobium species is rather limited. From a field survey conducted on the nodulation of *Vigna unguiculata* it was observed that effective rhizobium strains were present in soils of Sri Lanka (Mudannayake and Pain, 1986). The effect of combined nitrogen depends to some extent on host species and the strain of rhizobium.

In this study the effect of nitrogen on nodulation, N_2 fixation and dry matter production in the cowpea cultivar, MI—35, was examined.

MATERIALS AND METHODS

Cowpea plants (cultivar, MI—35) were grown in a growth chamber that provided 25°/22° C day and night temperature and 14-hour photoperiod with a photosynthetic photon flux density of 500 $\mu E/sq.m/sec$ at mid plant height (measured with a LI—190 Quantum Sensor and LI—185 Quantum meter, Lambda Instruments Company, USA) supplied by fluorescent and incandescent lamps. Seeds were surface sterilized with 0.1% mercuric chloride and germinated on sterilized filter paper in Petri-dishes under sterile conditions in the growth chamber. Seedlings were transferred to treatments in modified Leonard-jar assembly containing sterile vermiculite and N_2 free basic nutrient solution (Gibson, 1980). Potassium nitrate (KNO_3) was added to the basic nutrient solution and diluted to one fifth of the volume to prepare

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nitrate treatments of 0.0, 0.5, 1.0 and 2.0 mM concentrations. The initial pH of the medium was adjusted to 6.5. Treatments were replicated three times with two plants in each replication. Rhizobial strains CIAT 301 (obtained from Dr. Ivan A. Casas, Facultad de Ciencias Veterinarias, Universidad del Zulia, Maracaibo, Venezuela) and SLM 602 (obtained from the rhizobium culture collection, Microbiology Unit, Soybean Food Research Centre, Gannoruwa, Sri Lanka) were cultured on yeast galactose broth medium (Vincent, 1970) in 300 ml "Bellco" side-arm flasks on a horizontal shaker at 27°C. Rhizobial growth was estimated by measuring culture turbidity with a Klett-Summerson photoelectric colorimeter with a blue 400—465 nm filter (Dazzo *et al.*, 1976). Seedlings were inoculated at transplanting with 0.2 ml of six-day old rhizobial cultures grown in yeast galactose broth.

Plant dry weight, weight and number of nodules, and N_2 fixation by acetylene reduction method (Turner and Gibson, 1980) were measured for each plant after 4 weeks. Plants were shaken free from the vermiculite and sand, roots with attached nodules were detached, blotted dry and sealed in 50 ml pyrex test tubes with rubber stoppers. Five ml air was removed from each tube and replaced with 5 ml acetylene generated from calcium carbide. After one hour of inoculation at room temperature, a 5 ml gas sample was removed and injected into a 5 ml evacuated pyrex test tube. A 100 μ l gas sample was analyzed in a Perkin-Elmer Sigma 3B gas chromatograph, equipped with a Porapak N Column and carrier gas (N_2) flow rate set at 40 ml/min. The oven, injector and detector temperatures were 90°, 110° and 150° C respectively. Acetylene reduction rate was expressed as μ moles ethylene (reduced acetylene) per gram nodule fresh weight per hour.

RESULTS AND DISCUSSION

Nitrogen concentration and rhizobium strain affected the acetylene reduction activity significantly (Table 1). Acetylene reduction activity in the nodules reached the highest level at 1.0 mM KNO_3 concentration and the rhizobium strain CIAT 301 appeared to be more effective. In cowpea plants inoculated with rhizobium strain SLM 602,

Table 1. Effect of rhizobium strain and nitrogen level on nodule activity

	Nodule activity (μ moles ethylene/g f. wt/h)				
Rhizobium	Nitrogen level (mM KNO ₃)				Mean
Strain	0.0	0.5	1.0	2.0	
CIAT 301	46.1	19.6	89.9	60.5	54.0
SLM 602	22.4	15.2	44.9	46.6	32.3
Mean	34.2	17.4	67.4	53.5	
LSD Rhizobium strain (P = 0.05) = 5.5					
LSD Nitrogen level (P = 0.01) = 10.8					

the nodule activity reached a plateau, beyond 1.0 mM KNO₃ concentration whereas in the plants inoculated with rhizobium strain CIAT 301 the nodule activity was reduced beyond 1.0 mM KNO₃ concentration. The nodule activity reached the minimum level in both rhizobium treatments at 0.5 mM KNO₃ concentration. At 0.5 mM KNO₃ concentration a similar nodule activity was noted in both rhizobium treatments.

Nodule fresh weight was significantly affected by the nitrate concentrations tested. Nodule fresh weight reached its maximum at 1.0 mM KNO₃ concentration (Table 2). The pattern of nodule development showed a similar trend in both rhizobium treatments of CIAT

Table 2. Effect of rhizobium strain and nitrogen level on nodule fresh weight

		Nodule fresh weight (mg plant)				
Rhizobium		Nitrogen level (mM KNO ₃)				Mean
Strain		0.0	0.5	1.0	2.0	
CIAT 301		1139	1507	2462	2409	1879
SLM 602		1323	1466	2345	2220	1838
Mean		1231	1486	2403	2314	
LSD Nitrogen level (P = 0.01) = 265						

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301 and SLM 602 (Table 3). Rapid nodule development was observed between 0.5 mM and 1.0 mM KNO_3 concentrations and beyond 1.0 mM KNO_3 concentration there was no further nodule development. A significant variation in nodule number was recorded in both rhizobium and nitrogen treatments.

Table 3. Effect of rhizobium strain and nitrogen level on nodule number

		Nodule number/plant				
Rhizobium		Nitrogen level (mM KNO ₃)				Mean
Strain		0.0	0.5	1.0	2.0	
CIAT 301		110	145	181	121	139
SLM 602		144	172	226	244	196
Mean	127	127	158	203	182	
LSD Rhizobium strain (P = 0.01) = 10.8						
LSD Nitrogen level (P = 0.01) = 15.3						

Applied nitrogen in inoculated treatments resulted in a significant change in plant dry weight (Table 4). The dry weight of the plants

Table 4. Effect of rhizobium strain and nitrogen level on plant dry weight

		Dry Weight (g/plant)				
Rhizobium		Nitrogen level (mM KNO ₃)				Mean
Strain		0.0	0.5	1.0	2.0	
CIAT	301	1.62	2.40	4.46	5.35	3.46
SLM	602	1.94	2.55	4.47	5.53	4.83
Mean		1.78	2.47	4.46	5.44	
LSD Nitrogen level (P = 0.01) = 0.33						

continued to increase with the increasing KNO_3 concentration. A rapid increase in plant dry weight was noted between 0.5 mM and 1.0 mM KNO_3 concentrations.

In the cultivation of the cowpea cultivar MI—35, nitrogen fertilizer application is practised without considering the amount of nitrogen fixed biologically by these legumes. Generally when legumes are supplied with nitrogen fertilizer the nodules produced remain small or ineffective. The cowpea cultivar MI—35 is a short duration crop. Early and efficient nodule development for rapid nitrogen fixation is important for this cultivar to contribute to the nitrogen economy in cultivation. In this study the results indicated that very low KNO_3 concentrations (0.5 mM) decreased nodule activity, nodule fresh weight and nodule number. Rhizobium strain CIAT 301 has increased the nodule activity and nodule fresh weight in comparison with SLM 602. SLM 602 has increased the nodule number and plant dry weight at the tested KNO_3 concentrations. Beneficial effects on nodule development and nodule activity could be obtained at 1.0 mM KNO_3 concentration. Also it appears that the activity of rhizobium strain varies with the external nitrogen concentration. This indicates the potential feasibility of the effective use of rhizobium strain and fertilizer nitrogen. These findings however, need verification under field conditions.

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REFERENCES

- Broomfield, E. S. P. and R. J. V. D. K. Rao. 1983. Studies on fast and a slow growing rhizobium spp — nodulating *Cajanus Cajan* and *Cicey arietinum*. Ann. Apl. Biol. 102: 485 — 495.
- Dart, F. J. and F. V. Mercer. 1965. The effect of growth, temperature, levels of ammonium nitrate and light intensity on the growth and nodulation of cowpea. Aust. J. Agric. Res. 16: 321 — 345.
- Dazzo, F. B., C. A. Napeli and D. H. Hobbell. 1976. Adsorption of bacteria to roots as related to host specificity in Rh-Clover symbiosis. Applied and Environmental Microbiology 32 (1): 166 — 171.
- FAO. 1984. Pocket manual on legums inoculants and their use. Food and Agriculture Organization of the United Nations, Rome, Italy.
- Gibson, A. H. 1980. Methods for legumes in glasshouse and controlled environment cabinets. In Methods for evaluating biological nitrogen fixation. Ed. F. J. Bergersen. pp. 146 — 184. John Wiley and Sons, New York, USA.
- Mudannayake, C. and A. Pain. 1986. Evaluation of nitrogen fixing ability of Sri Lankan rhizobium species. Tropical Agriculturist 142: 11 — 24.
- Pankhurst, C. E. and W. T. Jones. 1979. Effectiveness of lotus root nodules 111. Effect of combined nitrogen on nodule effectiveness and flavolan synthesis in roots. J. Exp. Bot. 30: 1109 — 1118.
- Truner, G. L. and A. H. Gibsen. 1980. Measurement of nitrogen fixation by indirect means. In Methods for evaluating biological nitrogen fixation. Ed. F. J. Bergersen. pp. 111 — 138. John Wiley and Sons, New York, USA.
- Vincent, J. M. 1970. A manual for the practical study of root nodule bacteria. Blackwell Scientific Publications, Oxford, UK.