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Nodule occupancy and competitive ability of selected root nodulating bacteria of *Mucuna bracteata*

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INTRODUCTION

Cover crops are established and maintained in rubber plantations for the purpose of conserving the soil and improving and maintaining the soil structure and fertility. Leguminous ground covers fix a sustained quantity of atmospheric nitrogen and thus reducing the use of nitrogenous fertilizer. In legumes, seed inoculation with nodulating bacteria is widely practiced for effective and early nodulation and maximum nitrogen fixation. The success of seed applied strains of nodulating bacteria to form nodules depends on their ability to colonise the soil and rhizosphere and then to form nodules in competition with indigenous strains.

Mucuna bracteata is a fast growing legume found in the north eastern regions of India. It is drought resistant and shade tolerant with high nitrogen fixing capacity (Kothandaraman, *et al.*, 1989) and is introduced as a cover crop in the rubber plantations of Kerala. When *Mucuna* seeds were inoculated with an effective isolate of *Bradyrhizobium* from Kerala prior to sowing, increase plant growth, subsequent biomass addition, reduction in the weed population and increased soil microbial population were observed (Mathew *et al*, 2003).

The objectives of the present study were to understand the nodule occupancy of two isolates of nodulating bacteria of *M. bracteata* isolated from soils of North East India and a Kerala isolate and to determine their competitive ability to occupy the nodules using intrinsic antibiotic resistant markers

MATERIALS AND METHODS

Three isolates of nodule forming bacteria of *Mucuna, viz.*, NE-2 (Agarthala, Tripura isolate), NE-4 (Tura, Meghalaya isolate) and RRII-M (Kottayam, Kerala isolate) from culture collections of Rubber Research Institute of India were used for the study. These isolates were screened for their intrinsic antibiotic resistance markers using five antibiotics, *viz.*, ampicillin, tetracycline, chloramphenicol, gentamycin and streptomycin at concentrations ranging from 10ppm to 175ppm by incorporating to yeast extract mannitol agar medium and inoculating with the cultures. Growth of the isolates after three days of incubation was observed and the highest concentration of each antibiotic at which growth occurred were recorded as the resistant level of that antibiotic.

Occupancy in the nodules of *Mucuna* plants by these isolates and their competitive ability were studied based on IAR markers. Their effect on plant growth was also recorded when the three isolates were inoculated singly and in combination. Germinated seedlings of *M. bracteata* were transplanted to polythene bags with garden soil and three ml of each bacterial culture (cfu 10^8/ml) were applied to the soil pit at the time of planting. For the combined inoculation one ml each of the three cultures were mixed and applied. Ten plants were maintained for each treatment. Control plants with no bacterial inoculation were also maintained. After three months plants were uprooted and recorded various growth parameters. Nodules of

plants from each treatment were surface sterilized with mercuric chloride (0.2%), crushed, diluted and plated in YEMA media with antibiotics at the selected resistant level for each isolate. Control plates without antibiotics were also maintained for each isolate for enumerating total nodule bacteria and percent occupancy of the inoculated bacteria were calculated. Nodules of combined inoculated plants were plated for checking nodule occupancy of the three isolates by using the IAR markers in separate plates.

RESULTS AND DISCUSSION

The isolates were generally less resistant to streptomycin and gentamycin and more resistant to chloramphenicol and rifampicin. Occurrence of multiple drug resistance with different levels of resistance to various antibiotics by *Rhizobium* was also reported by Gupta *et al.* (1983). The resistance level of antibiotics selected for each isolate as markers were $Strep_{75}$ Rif_{150} for RRII M, Gen_{75} Chl_{150} for NE 2 and Tet_{125} Rif_{150} for NE 4.

The results of the single and mixed inoculation of the three bacterial isolates are given in table 1. Inoculation of the *Mucuna* plants with the two north east isolates showed better shoot and root growth and nodulation than the plants inoculated with the Kerala isolate. The combined inoculation did not show any additional effect than their single inoculation.

Table 1. Effect of inoculation of *M. bracteata* with different nodulating bacteria.

Inoculation	Shoot length (cm)	Shoot wt (g)	Root length (cm)	Root wt (g)	Root volume (ml)	No. of nodules	Wt. of nodules
RRII M	257.00	5.640	29.20	0.674	14.00	12.60	0.260
NE2 (Agarthala)	250.00	7.440	29.33	0.800	16.65	13.33	0.336
NE 4 (Tura)	327.25	7.022	24.00	0.765	13.75	14.50	0.415
NE2+NE4+RRIIM	224.60	5.156	33.20	0.624	14.00	10.20	0.228
Control	214.00	5.000	25.20	0.606	13.00	10.00	0.206
CD (P=0.05)	27.25	1.62	4.12	0.132	1.18	3.36	0.154

The occupancy in the nodules when the isolate were inoculated separately were 86, 82 and 96 per cent, respectively for RRII M, NE 2 and NE 4. The plants were also got infected by indigenous bacteria. When mixed inoculum was used, one of the strains was dominated and no definite trend of their competitive efficiency was noticed. Out of the ten plants, three were infected with the isolate RRII M, four plants each with the isolates NE 2 and NE 4. Only one plant showed mixed infection with the isolates RRII M and NE 4. Studies conducted by Nguyen *et al* (2010) also showed that in soybean cultivar Sachiyataka, plural strains belonging to distinct groups were obtained frequently from single nodules, indicating that multiple occupancy was established at high frequency and no fixed combination of the groups was found in the compositions of multiple occupancy.

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