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Isolation and Testing of Symbiotic Effectiveness of Rhizobia for *Pueraria phaseoloides* and *Calopogonium mucunoides*

GEORGE V. THOMAS AND M. V. SHANTARAM
Division of Microbiology, Central Plantation Crops Research Institute, Kayangulam-690 533

ABSTRACT

Isolates of *Rhizobium* sp. obtained from eight different forage legumes were tested for their nodulation and nitrogen fixing efficiency on *Pueraria phaseoloides* and *Calopogonium mucunoides* in Leonard jar assemblies. Both the legumes were promiscuous in their rhizobial requirement. Two efficient isolates were recognised which were further tested for their efficiency under field conditions along with two composite cultures from NifTAL. The NifTAL cultures were effective in nodulating and increasing the dry matter production in *C. mucunoides*. *P. phaseoloides* was effectively nodulated by native soil rhizobia and hence did not respond to rhizobial inoculation.

THE suitability of growing *Pueraria phaseoloides* and *Calopogonium mucunoides* in coconut basins for green manuring was demonstrated in recent experiments at the Central Plantation Crops Research Institute, Kayangulam, India (Thomas and Shantaram, 1984). Nodulation of these legumes with effective rhizobia helps in increasing the contribution of legume nitrogen and dry matter to the soil/plant system. Seed inoculation has become an established practice for cultivating legumes not nodulated by effective native soil rhizobia (Subba Rao, 1976). A number of isolates of rhizobia were obtained from local soils and tested for their ability to nodulate the two forage legumes, under glass-house conditions in Leonard jars. The performance of promising isolate was further tested under field conditions and the efficiency was compared with composite cultures obtained from NifTAL, Hawaii, U.S.A. The results are presented in this communication.

MATERIAL AND METHODS

Isolates of *Rhizobium* were obtained from naturally nodulated forage legumes (Table I) growing in potted soils. The nodular contents were streaked on to plates of yeast extract mannitol agar with congo red (Vincent, 1970). The plates were incubated at room temperature (25-30°C) for 6-10 days, single colonies were isolated and purified. The isolates were subjected to morphological tests and confirmation was based on their growth on Hofer's alkaline broth (Hofer, 1935) and the Ketolactase test (Bernaertz and Deley, 1963).

Glass house tests : The symbiotic relationships of eight of these strains were tested under glass house conditions with *Calopogonium mucunoides* Desv. and *Pueraria phaseoloides* (Roxb.) Benth. Testing units were Leonard jar assemblies containing sterilized washed and nitrogen-deficient nutrient solution (Norris, 1964). The seeds were surface sterilized with 0.1 per cent $HgCl_2$ for five minutes, washed thoroughly with sterilized water and transferred to sterile filter paper in petri dishes for germination. Four uniformly germinated uncontaminated seeds were sown in each Leonard jar. Rhizobial

inoculation was done two weeks after sowing by pipetting one ml cell suspension grown in mannitol yeast extract broth, containing approximately 10^9 cells per ml. Four replicate jars were used for each *Rhizobium* culture-legume variety treatment. Ten weeks after sowing, the plants were removed carefully, roots washed free of sand, the nodules detached and counted. The plant samples were dried at 70°C and weighed.

Field inoculation test : Two *Rhizobium* strains effective in nodulating the two host plants in the Leonard jar tests were further tested under field conditions. In addition, two composite cultures of *Rhizobium* obtained from NifTAL, Hawaii, U.S.A. were also used. These two cultures were (1) a *Centrosema* culture with a combination of three strains *viz.*, TAL S 304, 310 and 658 and (2) a *Stylosanthes* culture with TAL S 651, 655 and 1146. The experiment was laid out in the interspaces of a coconut garden in a sandy soil. Fifty plots of 2 m^2 size were prepared and were separated from each other by a distance of at least one m. Each treatment was replicated five times. Lignite based cultures of the local isolates of rhizobia were prepared from YEM broths having 10^9 cells/ml by mixing 50 ml of culture broth with 100 g of finely powdered lignite containing 8 g $CaCO_3$. Peat based NifTAL cultures were used as supplied. The seeds with the appropriate rhizobial treatment were sown in rows in the plots during June, 1982. The distance between row to row and plant to plant was 50 and 25 cm, respectively. After 4 months, five plants were harvested from each plot and nodule counts recorded. Nodule and plant dry weights were obtained after drying at 70°C for four days. The nitrogen content of plant samples was estimated by the micro-Kjeldahl method (Bremner, 1960).

Results and Discussion

Eight bacterial isolates from the nodules of native and exotic forage legumes grown in local soils were confirmed as rhizobia based on morphological and physiological tests. The data on relative effectiveness of local rhizobial isolates on *C. mucunoides* and *P. phaseoloides* are presented in Table I. A range of effectiveness among the rhizobial isolates was evident from the present study. The rhizobial inoculants also varied in their effectiveness with the two host plants. The best symbiotic efficiency in the case of *C. mucunoides* was by an isolate (No. 4) from *Desmodium intortum* based on plant weight and nodulation while in terms of nodulation alone an isolate from *Glycine weightii* (No. 6) was better. Similarly, isolate No. 4 obtained from *D. intortum* nodulated better and isolate No. 5 obtained from *Macrotyloma axillaire* nodulated and also produced maximum dry matter yield in *P. phaseoloides*. The isolates from *Macroptilium atropurpureum* and *Desmodium uncinatum* were not effective on either of the hosts, whereas an isolate from *Mimosa pudica* was not effective on *Pueraria*. It was interesting to note that rhizobial cultures isolated from other hosts performed better with both legumes than the isolates obtained from them.

Broughton *et al.* (1975) recorded similar variations in symbiotic effectiveness of *Rhizobium* in *Centrosema pubescens* under Leonard jar conditions. Allen and Allen (1939) also recorded a range of variation in effectiveness of rhizobia with *P. phaseoloides*. Quintero and Garza (1978) reported that a rhizobial isolate from *P. phaseoloides* had varying nodulating efficiencies on *Centrosema pubescens*, *Clitoria ternata* and *Macroptilium atropurpureum*. Eventhough *Rhizobium* from *Glycine weightii* produced maximum number of nodules on *C. mucunoides*,

TABLE I

Nitrogen fixing efficiency of local rhizobial isolates under controlled glass house conditions

Isolate No.	Source (Host from which isolated)	*Calopogonium mucunoides* Nodule* number	Plant dry wt. (g)	*Pueraria phaseoloides* Nodule number	Plant dry wt. (g)
	Uninoculated control	0.00	0.432	0.00	0.380
1.	*Pueraria phaseoloides*	22.00	0.420	7.00	0.640
2.	*Calopogonium mucunoides*	22.50	0.686	3.50	0.420
3.	*Mimosa pudica*	44.25	0.300	0.00	0.256
4.	*Desmodium intortum*	54.00	1.396	9.25	0.704
5.	*Macrotyloma axillaire*	20.00	0.552	6.00	1.040
6.	*Glycine weightii*	128.50	0.360	3.25	0.300
7.	*Macroptilium atropurpureum*	0.00	0.340	0.00	0.364
8.	*Desmodium uncinatum*	0.00	0.480	0.00	0.272
	C. D. at 5%	NS	0.143	3.50	0.109

Values represent average of four replications.

*Statistical analysis done after $\sqrt{(X+0.5)}$ transformation of actual values.

there was a decrease in plant dry weight indicating the non-effective nature of the nodules formed and they have even exhibited parasitic relationship with the host.

Both the hosts were promiscuous with regard to their rhizobial specificity. *C. mucunoides* was nodulated by six and *P. phaseoloides* by five of the eight isolates tested. Symbiotic promiscuity of different hosts in cowpea group is well established (Allen and Allen, 1939; Norris, 1964). Ikram and Broughton (1980) in a similar study observed that 13 out of 14 rhizobial isolates from different genera of legumes effectively nodulated *Psophocarpus tetragonolobus*. Allen and Allen (1981) have stated that the rhizobia nodulating the two legumes belong to the cowpea miscellany group. Because of their relative effectiveness isolate Nos 4 and 5 were selected for further testing under field conditions.

The field performance data on nodulation, dry matter production and nitrogen content in the two hosts inoculated with local isolates of rhizobia (Nos. 4 and 5) and NifTAL inoculants are presented in Table II. *C. mucunoides* responded significantly to inoculation with NifTAL cultures. Local isolate No. 4, which was found to be very effective under Leonard jar conditions, failed to perform well under field conditions. With regard to *P. phaseoloides*, statistical analysis of the data revealed that none of the rhizobial inoculants were effective eventhough some increased nodulation and dry matter yield was recorded with isolate No. 4 and the NifTAL culture. Isolate No. 5 which was effective on *Pueraria* under Leonard jar conditions failed to perform well under field conditions.

Among the four inoculants tested in the field, a significant response to inoculation was obtained from NifTAL cultures only with *C. mucunoides*. The variable response in the Leonard jar tests and field perfor-

TABLE II

Field response to rhizobial inoculation in Calopogonium mucunoides *and* Pueraria phaseoloiees *in a sandy soil*

Treatment	*C. mucunoides*				*P. phaseoloides*			
	Nodule Number	Nodule dry wt. (g)	Plant dry wt. (g)	Nitrogen content (g)	Nodule number	Nodule dry wt. (g)	Plant dry wt. (g)	Nitrogen content (g)
Local isolate No. 4	35.0	0.113	9.3	0.190	44.2	0.405	17.5	0.364
Local isolate No. 5	36.8	0.090	11.0	0.200	37.4	0.242	15.5	0.251
NifTAL *Stylosanthes* culture	80.4	0.187	15.4	0.320	47.0	0.387	18.4	0.380
NifTAL *Centrosema* culture	67.2	0.250	15.6	0.330	48.8	0.260	12.0	0.259
Control	29.0	0.097	10.5	0.160	26.4	0.225	12.3	0.235
C.D. at 5%	33.9	0.101	4.6	0.078	NS	NS	NS	NS

Values represent average of five replications.

mance of the rhizobial isolates demonstrate the effect of both biological and non-biological factors under natural conditions in modifying the expected response (Lie, 1974).

The nodules of *P. phaseoloides* in uninoculated treatments were deemed to be effective because of their bold appearance and pink internal pigmentation. This nodulation by indigenous strains is probably the principal reason for non-significant response to inoculation. It is also significant that in these soils cultivation of legumes is not practiced, but the rhizobia capable of nodulating exotic plant species like *Pueraria phaseoloides* and *Calopogonium mucunoides* still occur. Weaver *et al.* (1972) recorded populations of *Rhizobium japonicum* in soils where soybean was not grown for the previous 13 years. This indicates the capacity of native soil rhizobia to saprophytically survive in soils over long periods even in the absence of specific host plants. It is concluded from these studies that NifTAL composite cultures recommended for *Centrosema* and *Stylosanthes* performed well on *Calopogonium mucunoides* in these sandy loam soils. The native soil rhizobia nodulated *P. phaseoloides* effectively thus indicating that there may not be need to inoculate the host with specific rhizobial inoculants.

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