RHIZOBIUM REQUIREMENTS FOR TWO ACCESSIONS OF *GLIRICIDIA MACULATA*

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ABSTRACT

Gliricidia maculata *has been difficult to nodulate when sown in the field in southern Queensland. Newly isolated strains of* Rhizobium *from nodules of* G. maculata *were compared with strains CB905 (from* Sesbania*) and CB756 (a wide spectrum strain from* Macrotyloma*). The only effective strains were from* G. maculata. *Strains CB3049 and CB3092 were the most effective in nitrogen fixation on an accession of* G. maculata *from Bali, whereas on a West Indian accession strains CB3057 and CB3090 were the most effective. These four strains were used to inoculate the Bali accession sown into soil in a glasshouse pot experiment. The most effective strain (highest plant and nodule dry weight in soil) was CB3090 and it is recommended as a potentially useful inoculum strain for this species.*

INTRODUCTION

Gliricidia maculata is a fast growing, medium sized leguminous tree commonly used as shade for coffee, cocoa and tea. However, it is also used as a forage tree (Kantharaju and Chadhokar 1981; Falvey 1982). In growing this plant in southern Queensland we have observed that it does not nodulate effectively with the wide spectrum cowpea-type strain CB756, and only sparsely with *Rhizobium* present in the soil. Swarbrich (1964) observed that when *G. maculata* was being used as a shade for cocoa there were no nodules present. This paper reports an experiment designed to evaluate nitrogen fixation effectiveness of strains of *Rhizobium* on *G. maculata* growing in nutrient solution and in soil.

MATERIALS AND METHODS

Nutrient solution—Rhizobium effectiveness in pure culture

Sand-jar assemblies were used to compare the effectiveness of a range of strains of *Rhizobium* on *G. maculata*. The sand-jar contained washed and sterilized sand and a nitrogen free nutrient solution as described by Norris and Date (1976).

Seed was surface sterilized in 0.1% acidified $HgCl_2$ solution for 2 minutes, washed and pre-germinated on 1% water agar plates at 25 °C. Uniformly germinated seedlings were selected and two were sown into each sand-jar assembly. Seedlings were inoculated 4 days after sowing by pipetting 1 ml of a 10^9 ml^{-1} suspension of *Rhizobium* onto each jar. Details of the strains of *Rhizobium*, host and country of origin are given in Table 1. In addition there was an uninoculated control with no nitrogen and one with nitrogen added at the rate of 10 mg N jar^{-1} (as NH_4NO_3) on days 0, 27, 39 and 44 after planting. Plants were sown on February 4, 1982 and harvested April 2, 1982. There were 4 replications per treatment and two accessions of *G. maculata*, one from Bali and one from the West Indies.

Soil experiment—pot trial in the glasshouse

From the 15 strains tested in the sand-jar assemblies, strains CB3057, CB3059, CB3090 and CB3092 were chosen to compare with uninoculated and plus nitrogen treatments in a soil-pot experiment. A gleyed podzolic soil (not previously sown with *Gliricidia*) from the Mt. Cotton Research Station (27.7 °S, lat., 152 °E long.), fertilized with P, K, S, Cu, Zn and Mo at levels recommended by Wallis *et al.* (1977), was used with 1900 g of air-dried soil in a 15 cm pot. Seeds of the Bali accession were surface sterilized, germinated and a single seedling transplanted to each pot. We could not use

TABLE 1

Strains of Rhizobium, *their growth rates and origins*

Strains*	Rhizobium Growth rates	Origin	
		Country	Plant
CB3057, CB3083, CB3084	Fast growing	Australia	*G. maculata*
CB3051, CB3085, CB3086, CB3087	Fast growing	Solomon Islands	*G. maculata*
CB3088, CB3089, CB3090, CB3091, CB3092	Fast growing	Sri Lanka	*G. maculata*
CB756	Slow growing	Kenya	*Macrotyloma africanum*
CB905	Fast growing	U.S.A.	*Sesbania macrocarpa*
CB3082	Fast growing	Botswana	*Sesbania* sp.

* Strains of *Rhizobium*, where there are more than one in a group, were isolated from different nodules collected from the same plant.

the West Indian accession because of lack of seed. As in the sand-jar experiment seedlings were inoculated with 1 ml of a 10^9 ml^{-1} suspension of *Rhizobium*. Nitrogen was added in solution to the plus nitrogen control treatment as 10 ml of a 1 mg ml^{-1} solution of NH_4NO_3 before sowing. Each treatment was replicated four times in a completely randomized design. The pots were sown on July 14, 1982, located in a temperature controlled glasshouse (30/25°C day/night), and plants harvested on October 6, 1982.

Tops of plants were cut and oven dried. Roots were washed out, nodules collected and counted, and both were oven dried and weighed. Tops were analysed for nitrogen and phosphorus by a Kjeldahl digestion followed by an auto-analysis technique (Oweczkin and Kerven 1980).

RESULTS

Nutrient solution trial—dry matter yields and nodulation

Twelve of the 15 strains tested formed effective associations which yielded significantly more than the uninoculated control, strains CB905 and CB3082 from *Sesbania*, and the wide spectrum strain CB756 (Table 2). The largest differences between accessions were with strains CB3090 and CB3091. Both of these strains were in the top group of strains for the West Indian accession but neither one occurred in the top group for the Bali accession.

The most effective strains on the Bali accession were CB3059 and CB3092, although six of the strains of *Rhizobium* increased the relative dry matter yield to greater than 100% of the plus nitrogen control (Table 2).

For the West Indian accession the best strains were CB3057 and CB3090 and 10 of them yielded more than 100% of that of the plus nitrogen control (Table 2).

All strains formed nodules but for strains CB756, CB905 and CB3082 nodules were small and fewer than 10 per plant. Only 50% of the plants of the Bali accession and 50%, 25% and 0% of the West Indian accession were nodulated from these three strains.

Soil experiment

Dry matter yield

The strains of *Rhizobium* selected for this experiment were the two highest yielding for each of the two accessions (Table 3). All strains gave lower yields (P = 0.05) than the plus nitrogen control treatment. Strains CB3090 and CB3092 gave higher yields (P

TABLE 2

Nutrient solution trial comparing the effect of strains of Rhizobium *on total dry matter yield (g plant^{-1}) and relative yield of the Bali and West Indian accessions of* G. maculata

Strains	Total D.M. Yield		Relative Yield	
	Bali	West Indies	Bali	West Indies
	(g plant^{-1})		(% of +N control)	
CB3059	6.64^{a}	4.88^{abc}	132	119
CB3092	6.63^{a}	4.39^{abcd}	131	107
CB3083	6.19^{a}	3.78^{d}	123	92
CB3057	6.01^{ab}	5.11^{ab}	119	125*
CB3084	5.78^{ab}	4.54^{abcd}	115	111
CB3088	5.65^{ab}	4.13^{abcd}	112	108
Control +N	5.04^{bc}	4.09^{bcd}	100	100
CB3090	4.98^{bc}	5.43^{a}	99	133*
CB3086	4.96^{bc}	4.32^{bcd}	98	106
CB3089	4.96^{bc}	3.93^{cd}	98	96
CB3087	4.95^{bc}	4.32^{bcd}	98	106
CB3085	4.71^{bc}	4.68^{abcd}	93	114
CB3091	4.21^{c}	4.89^{abc}	83	120
Control -N	1.49^{d}	1.31^{e}	30	32
CB3082	1.39^{e}	1.18^{e}	28	28
CB756	1.32^{e}	1.42^{e}	26	35
CB905	1.30^{e}	1.44^{e}	26	35

*The two highest yielders for the West Indian accession. Means sharing a common letter are not significantly different at P = 0.05.

TABLE 3

Pot trial in soil on the effects of inoculation of the best strains of Rhizobium *from the Bali and West Indian accessions in the nutrient solution trial on the nodule and total plant dry weight of the Bali accession.*

Strains	Nodule Dry Wt.	Total Dry Wt.	Relative Yield
	(g plant^{-1})	(g plant^{-1})	(% of +N Control)
Control +N	—	7.99^{a}	100
CB3090	0.178^{a}	6.79^{b}	85
CB3092	0.158^{ab}	6.52^{b}	82
CB3059	0.139^{b}	5.73^{c}	72
Control—N	0.158^{ab}	5.69^{c}	71
CB3057	0.148^{b}	5.39^{c}	67

Means sharing a common letter are not significantly different at P = 0.05.

= 0.05) than CB3057, CB3059 and the uninoculated control. The uninoculated control was well nodulated and gave higher (but not significantly) yields than CB3057.

Nodulation

Strain CB3090 which gave the highest plant dry weight yield also had the highest nodule dry weight per plant (Table 3), but was not significantly different from CB3092 or the uninoculated control. Strains CB3057 and CB3059 had lower nodule dry weights than CB3090.

Nitrogen and Phosphorus Concentration of Tops

The nitrogen and phosphorus contents of tops for all treatments, including both controls, were 2.80 to 2.99% and 0.13 to 0.15% respectively except for strain CB3092 which had lower levels at 2.41% and 0.11% respectively.

DISCUSSION

Only the strains of *Rhizobium* isolated from *Gliricidia*, regardless of country of origin, formed effective nitrogen fixing associations with the two test accessions of

Gliricidia maculata. There was an interaction between strains of *Rhizobium* and accessions of *G. maculata*. The two highest yielding strains on the Bali accession were different from the two highest yielding strains on the West Indian accession. Similar interactions have been observed previously between strains of *Rhizobium* and genotype of host (e.g., Date 1977; Caldwell and Vest 1977). However, these relationships did not apply when these four strains were used to inoculate the Bali accession of *G. maculata* in the soil experiment. Only one strain from each of the two pairs of highest yielding strains gave yields better than that of the uninoculated N-free control. Both of these strains, CB3090 and CB3092, originated from the same nodule sample from Sri Lanka. It is possible that these strains were more competitive for nodule formation with the existing soil strains than were the other two strains which originated from nodule samples obtained in Australia and the Solomon Islands. If this is so then the higher yields would be due to the formation of more nodules of the inoculum strains which were more effective in nitrogen fixation than the existing soil strains. Soil factors such as pH, organic matter, levels of aluminium, manganese, phosphorus and competition with other soil organisms are known to differentially affect the success of introduced strains of rhizobia (e.g., Caldwell and Vest 1970; Ham *et al.*, 1971; Date and Brockwell 1978). Although proportions of nodules due to each strain were not determined it is possible in the strain CB3057 and CB3059 treatments that no nodules of these strains were formed. On the assumption that the native soil strains also were poorly effective, it may be that soil N accounted for the plant growth in the uninoculated, CB3057 and CB3059 treatments.

The lower nitrogen and phosphorus content of tops of plants inoculated with CB3092 (99 mg N plant^{-1}) than for those inoculated with CB3090 (125 mg N plant^{-1}) suggests that strain CB3090 may be a more efficient fixer of nitrogen than is CB3092 (Caldwell 1969; Ham *et al.* 1971).

Further evaluation of strains of rhizobia and the need for inoculation of *G. maculata* grown in the field is warranted. However, strain CB3090 appears useful and is recommended for use with the Bali accession.

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