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INCREASED EFFECTIVENESS OF COMPETITIVE RHIZOBIA STRAINS UPON INOCULATION OF CAJANUS CAJAN

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ABSTRACT

A field study was conducted in lysimeters containing ^{15}N enriched soil to determine the effects of 4 competitive rhizobial strains upon yield parameters of pigeon peas (Cajanus cajan). The greatest differences observed were in seed yields: strain P 132 effected the highest (121 ± 20 g/plant), and the control (indigenous rhizobia) effected the lowest (43.9 ± 8 g/plant). With the exception of seeds and pods, the dry matter weight was not different. Although there appeared to be no effect of inoculum strains on the fractional content of N derived from biological nitrogen fixation (BNF) when the total plant biomass was considered, strains P 132 and 401 partitioned more of the N derived from fixation into seeds and leaves than the other strains. Because the seeds comprised the major portion of plant N, more total N and more derived from BNF (about half) were found in plants inoculated with P 132, while the smallest amount was found in the uninoculated controls. P 132 was also the best competitor with respect to indigenous rhizobia, and accounted for all the nodules found from the plants in which it was inoculated.

KEY INDEX WORDS: ^{15}N, nitrogen fixation, legumes, tropical soils, pigeon peas

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INTRODUCTION

Cajanus cajan L. Millsp. (pigeon peas) are commonly nodulated by the cowpea miscellany group of rhizobia, which are indigenous in tropical soils. Nitrogen fixation is often limited if the natural population is ineffective at promoting high yields. Thus, it may be necessary to replace the existing rhizobia by introducing more effective strains, but this is possible only if the inoculum strain can compete successfully against indigenous rhizobia. Although several investigators have been mainly concerned with the ability to compete for nodule sites (2,4,11), the capacity to fix a significant amount of nitrogen is a criterion that is equally as important.

In a recent greenhouse study with ^{15}N, El Hassan and Focht (7) showed that three of six rhizobial strains were good competitors, by comparison to the indigenous rhizobia, and also increased seed yield, shoot dry matter, total N and percentage N derived from fixation. Although ^{15}N has been used to measure N_2 fixation in legumes, few studies have evaluated the importance of strain effects on biological nitrogen fixation. Only two ^{15}N studies performed in the greenhouse, have alluded to the possible importance of strain effect upon partitioning of nitrogen to and within the plant (7,12).

The following study was undertaken to determine 1) if superior inocula strains (with respect to indigenous rhizobia) were competitive under field conditions; and 2) if different rhizobial strains can influence the plant partitioning of biologically fixed dinitrogen.

MATERIALS AND METHODS

Rhizobium Strains. Sources and physiological characteristics of rhizobial strains P 132, IHP 147, 401, and 22A1 have been described previously (7,10). The four strains were intrinsically resistant to rifampicin (150 μg/ml), erythromycin (100 μg/ml), and nalidixic acid (150 μg/ml).

Media and Inoculant Preparation. Strains were maintained on yeast extract mannitol (YEM) agar slants (10). Liquid cultures were grown in side arm flasks (250 ml) containing 50 ml of YEM broth on a rotary shaker platform (125 rpm) at 28°C to an optical density of 0.6 (525 nm). Seeds of Cajanus cajan (L.) Millsp. var. 64-2b (pigeon peas) were inoculated with a peat-based inoculant which was prepared by mixing 25 g of sterile peat (Nitragin Co.) with 100 ml of a liquid culture to obtain a final rhizobial count of 10^8/g peat.

Chemicals. Antibiotics (rifampicin, nalidixic acid, and erythromycin) were obtained from Sigma Chemical Co., St. Louis, MO. $(^{15}NH_4)SO_4$ (99.07 atom. % ^{15}N) was obtained from Biorad, Richmond, CA.

Field Studies. Cajanus cajan was grown at the field station of the University of Panama, Tecumen, R.P. Plants were grown in 55-gallon lysimeters, which had a surface area of 0.196 m, a depth of 100 cm, and a drain at the bottom. Each lysimeter contained one plant, and the distance between plants was 50 cm. The soil contained in the lysimeters was classified as a mixed, hyperthermic Tropecatic Haplorthox. ^{15}N enriched wheat straw (10 atom % ^{15}N) from a previous study (9) was

added to the soil three years prior to the field study to give an organic ^{15}N enrichment of 0.400%. At planting, $^{15}(NH_4)_2SO_4$ was added in sufficient amount to increase the enrichment to 0.430% ^{15}N. Treatments consisted of inoculated (P 132, IHP 147, 22A1, and 401) and uninoculated (control) plants, which were each replicated 4 times. *Eucalyptus* sp. was used as a non-leguminous control plant to measure the available ^{15}N content of the soil. Plants were grown to maturity and plant parts (leaves, pods, seed, and shoot) were harvested separately and dried at 68°C for a period of 48 hours. Nitrogen contents of plant material were determined by the procedures of Bremner and Mulvaney (3). ^{15}N mass spectrometric analyses of plant and soil samples were determined by Isotope Services, Inc. (Los Alamos, NM).

Identification of Rhizobial Strain in the Nodule. Antibiotic typing of nodules was used to determine the rhizobial strain present in the nodules. After the plants were harvested, the roots were dug up and attached nodules were collected and washed free of soil. Replicates within each treatment were pooled together, and the nodules were prepared for typing by surface sterilization in 95% ethanol (30s), followed by treatment with 0.2% acidified HgCl for 4 min and rinsing in sterile distilled water. Each nodule was crushed with a sterile glass rod, and the resulting bacteroid suspension was streaked on YEMA plates containing erythromycin (50 μg/ml), rifampicin (100 μg/ml) and nalidixic acid (100 μg/ml). Cultures were incubated at 28°C for 6 days. Infection by the inoculum strain was noted by growth on the plates.

RESULTS

The atom % ^{15}N of Kjeldahl digests of soil was 0.410 ± 0.004 for 25 samples of duplicated analyses. The atom % ^{15}N from the 4 samples of Eucalyptus leaves (0.437 ± 0.027) and that from 4 samples of shoots (0.424 ± 0.027) were not significantly different and were pooled together to use the value of 0.431 ± 0.021 for the available N content of the soil. The fractional content of N derived from biological nitrogen fixation (BNF) was determined by the isotope dilution procedure of McAuliff et al. (14). Further discussion of this procedure can be found in the recent review by Chalk (5).

There was no difference in dry matter yields of leaves and stems among inoculum strains (Table 1), but there was considerable difference in seed yield between the uninoculated and inoculated plants. The % N content was unaffected by the inoculum strain (Tables 1 and 2). The most significant difference effected by inoculation was upon seed yield (Table 1), which was highest with P 132 (121 g/plant) and lowest with the control. Consequently, a significantly greater amount of total N among inoculum treatments was noted: P 132 was the highest, and the uninoculated control was the lowest (Table 2). Nitrogen fixation was also significantly higher in plants inoculated with P 132.

Although there was no inoculum effect upon ^{15}N enrichment of the total plant, there was a difference in ^{15}N content among the plant components (Table 3). This difference was most pronounced with strain P 132,

which had the highest proportion of N derived from BNF (i.e., the lowest ^{15}N enrichment in the most active parts of the plants, namely the leaves and seeds). The partitioning effect with strain 401 was less pronounced but still statistically significant. Despite the lack of high statistical significance ($P < 0.05$) with strains 22A1 and IHP 147, the F values are statistically higher than the control at $P < .10$.

Rhizobia strain inoculations were successful as evidenced by the high proportion of antibiotic resistant rhizobia in the nodules (Table 4). Although all nodules were not recovered due to senescence, sloughing, and mechanical disruption after harvest, more intact nodules were found among P 132-inoculated plants than among others. P 132 was the most competitive strain since it formed all of the nodules typed (Table 4).

DISCUSSION

Although all strains competed successfully in comparison to the indigenous rhizobia, not all were equally effective. This is clearly shown by comparing P 132 and 22A1. While 22A1 performed well as a competitor, it did not fix as much nitrogen or effect as great a seed yield as P 132. The superiority of strain P 132 in terms of both competitiveness and effectiveness has also been shown in previous experiments (7,8).

Strains P 132, and to a lesser extent 401, were able to influence the partitioning of fixed nitrogen to the seeds and leaves. The partitioning of fixed N_2 to the more valuable and important parts of the plant by rhizobium strains should be an important selection criteria.

Strain P 132 provided approximately half of the plant nitrogen, while strains 401, 22A1, and IHP 147 fixed considerably less. The quantity of nitrogen fixed by P 132 is comparable to that reported for soybeans (16), yet lower than that obtained for cowpeas (7). According to a previous study by La Favre and Focht (12), however, pigeon peas fixed 91-94% of the nitrogen in the plant. The difference in the amount of nitrogen derived from fixation, was most probably due to the higher organic content of the soil used in this study (5.0%) in comparison to the other [0.5%; (12)]. Nevertheless, the total amount of biologically fixed nitrogen in our study (150 kg N/ha) is comparable to that calculated by La Favre and Focht (12).

P 132 increased seed yield significantly in comparison to uninoculated plants. Strains 401 and IHP 147 also effected higher yields in terms of increases in pods and seeds. Yield performance by strain P 132 had been demonstrated in earlier experiments performed under axenic conditions in Leonard jars (13) and in the greenhouse (12). Seed yields obtained with this inoculum strain, however, are considerably higher (2350 kg/ha) than those reported by La Favre and Focht (12) (825 kg/ha), Chowd-hury and Bhatia (6) (700-800 kg/ha), and Akinola et al. (1) (500-1500 kg/ha). Stem and leaf yields were, on the other hand, not increased by inoculation. The latter observation sheds doubt on the use of shoot biomass for assessment of strain effectiveness in biological nitrogen fixation.

Field inoculations of selected superior strains can successfully compete with less desirable indigenous strains. Superior strains may

also influence the partitioning of biologically fixed N, giving higher N contents in the more valuable parts of the crop plant. The selection of strains with both of these qualities would be valuable for biotechnological applications.

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Table 1. Dry matter yields (g) of *Cajanus cajan* (per single plant) as affected by inoculum strain.

Strain	Leaves	Pods	Seeds	Stems
P 132	23.1a[1]	70.0ab	121 a	90.5a
IHP 147	16.9a	59.7ab	91.8ab	77.0a
22A1	21.4a	52.7b	77.7b	71.5a
401	26.4a	71.4a	100 ab	106 a
Control	24.7a	46.9b	43.9c	90.5a

[1]Numbers followed by the same letter in each column are not significantly different by Duncan's Multiple Range Test ($P < 0.05$).

Table 2. Nitrogen content (g) of above-ground portion of plants after harvest.

Strain	Total N	BNF[2]
P 132	5.97a[1]	2.94a
IHP 147	4.59b	1.12b
22A1	4.37bc	1.03b
401	5.50ab	2.11ab
Control	3.14c	1.03b

[1]Numbers followed by the same letter in each column are not significantly different by Duncan's Multiple Range Test ($P < 0.05$).

[2]Biological Nitrogen Fixation as determined by ^{15}N isotope dilution: 1 g per surface area of lysimeter is equivalent to 51.0 kg/ha.

Table 3. Effects of inocula strains on partitioning of available soil nitrogen (0.431 ± 0.021) among plant parts of *Cajanus cajan*. Values are expressed in atom % ^{15}N.

Strain	Leaves	Pods	Seeds	Stems	F[1]
P 132	0.395a	0.414b	0.393a	0.421b	9.08**
IHP 147	0.402	0.426	0.417	0.427	2.17*
22A1	0.406	0.423	0.415	0.421	2.16*
401	0.392a	0.414b	0.403ab	0.411b	5.37**
Control	0.396	0.417	0.411	0.414	0.85NS

[1]*, ** significant at the 0.05 and 0.01 levels, respectively, and NS = not significant for analysis of variance among rows.

All values followed by the same letter in each row are not different ($P > 0.05$) from each other by Duncan's Multiple Range Test. ANOV comparison among columns of plant parts showed no significant difference at $P < 0.05$.

Table 4. Fractional nodule occupancy in *Cajanus cajan* based on intrinsic antibiotic resistance.

Inoculant Strain	Number of Nodules Sampled	Fraction of Antibioti Resistant Nodules
P 132	32	1.00
IHP 147	15	0.87
22A1	12	0.75
401	15	0.67
None (Control)	11	0.09