Symbiotic interactions of *Phaseolus acutifolius* and P. *acutifolius X P. vulgaris* hybrid progeny in symbiosis with *Bradyrhizobium spp.* and *Rhizobium leguminosarum* bv. *Phaseoli*[1]

PADMA SOMASEGARAN[2] AND HEINZ J. HOBEN

University of Hawaii, NifTAL Project, 1000 Holomua Avenue, Paia, HI 96779, U.S.A.

AND

LISA LEWINSON

Centro Internacional de Agricultura Tropicale (CIAT), Cali, Colombia

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The rhizobial requirements and the N,-fixing potential of *Phaseolus acutifolius* and P. *acutifolius X P. vulgaris* hybrid progeny were investigated in glasshouse experiments. Although P. *acutifolius* was promiscuous, effective nodulation and N_2 fixation occurred only with a few *Bradyrhizobium* isolates, especially those that were isolated from P. *acutifolius, P. limensis, P. penduratus, P. lunatus, Canavalia ensiformis, Calopogonium mucunoides,* and *Psophocarpus tetragonolobus.* Strong host X *Bradyrhizobium* interactions were detected in four genotypes of P. *acutifolius* tested against eight isolates of bradyrhizobia and a commercial mixed inoculant. All seven progeny tested nodulated with *Bradyrhizobium* isolates and *Rhizobium leguminosarum* bv. *phaseoli,* but there was a highly specific rhizobial requirement for effective nodulation and N_z fixation. The progeny X rhizobial interaction accounted for *83%* of the total phenotypic variation. Two (P-6 and *P-7)* and five (P-1, *P-2, P-3, P-4,* and *P-5)* progeny nodulated and fixed N_2 effectively with R. l. bv. *phaseoli* and *Bradyrhizobium* spp., respectively. The R. l. bv. *phaseoli* - progeny symbiosis had a greater N_2-fixing potential than the *Bradyrhizobium* isolate - progeny symbiosis. In a soil (oxisol) test, progeny P-6 and *P-7* showed significant response to inoculation with R. l. bv. phaseoli. Strain *R. 1.* bv. *phaseoli* TAL *182* was the most competitive strain, occupying *84%* of the nodules in both the progeny.

Key words: rhizobial requirements, *Phaseolus acutifolius* X *Phaseolus vulgaris* hybrids.

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Les exigences rhizobiennes et le potentiel de fixation de N_2 de *Phaseolus acutifolius* et des descendants hybrides de *P. acutifolius X P. vulgaris* ont ete etudies de fagon experimentale en serre. Bien que le P. *acutifolius* Wait pas ete discriminant, la production de nodosites et la fixation de N_2 n'ont eu de succes qu'avec quelques isolats de *Bradyrhizobium, plus* specifiquement ceux qui furent isoles de P. *acutifolius, P. limensis, P. penduratus, P. lunatus, Canavalia ensiformis, Calopogonium mucunoides* et *Psophocarpus tetragonolobus.* Une forte interaction h6te X *Bradyrhizobium* a ete decelee chez quatre genotypes de P. *acutifolius* testes en fonction de huit isolats de *Bradyrhizobium* et d'un inoculant mixte commercial. Les descendants des sept especes testees ont tous produit des nodosites avec les isolats de *Bradyrhizobium* et de *Rhizobium leguminosarum* bv. *phaseoli;* toutefois, les exigences rhizobiennes pour 1'efficacite de production de nodosites et de fixation de N_2 a ete hautement specifique. La descendance X 1'interaction rhizobienne est a 1'origine de *83%* de la variation phenotypique totale. Deux descendances (P-6 et *P-7)* et cinq autres (P-1, *P-2, P-3, P-4* et *P5)* ont produit des nodosites et on fixe efficacement le N_2, respectivement avec le R. l. bv. *phaseoli* et les *Bradyrhizobium* spp. La symbiose descendance -R. l. bv. *phaseoli* a eu un plus grand potentiel de fixation de N_2 que la symbiose descendance - isolats *Bradyrhizobium.* Dans un essai fait avec de l' oxisol, les descendants de P-6 et *P-7* ont repondu significativement a 1'inoculation avec le R. 1. bv. phaseoli. La souche R.l. bv. *phaseoli* TAL *182* a ete la plus competitive, occupant *84%* des nodosites chez les deux descendances.

Mots cles : exigences rhizobiennes, hybrides de *Phaseolus acutifolius* X *Phaseolus vulgaris.*

[Traduit par la redaction]

Introduction

Four agriculturally important species are normally recognized in the legume genus *Phaseolus* and these are *P. vulgaris* (common bean), P. *coccineus* (scarlet runner bean), *P. lunatus* (lima bean), and *P. acutifolius* (tepary bean) (Smartt 1970). Domesticated and cultivated genotypes of tepary have been found throughout much of North America and have been harvested for food by the American Indians (National Academy of Sciences 1979). Tepary is tolerant to drought, high temperatures, and poor soils (Chavan *et al.* 1965; Vietmeyer1986). In addition, some tepary genotypes are also resistant to common bacterial blight caused by *Xanthomonas phaseoli* (Coyne and Schuster 1973). These special traits of tepary are of special interest in bean breeding where the major objectives are to improve yield, nutritive value, disease resistance, and symbiotic N_Z fixation (Mok *et al.* 1986). Interspecific hybrids between P. *acutifolius* and *P. vulgaris* have been obtained, but they are normally sterile (Honma 1956; Smartt 1970). However, generations of fertile hybrids between these two species have been achieved by embryo culture (Mok *et al.* 1986) and by colchicine doubling (Prendota *et al.* 1982).

It is well known that the specific microsymbiont that nodulates *P. vulgaris, P. coccineus,* and *P. angustifolius* is *Rhizobium leguminosarum* bv. *phaseoli* (Fred *et al.* 1932; Jordan 1984).

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[2] Author to whom all correspondence should be addressed. Printed in Canada / Imprim6 an Canada

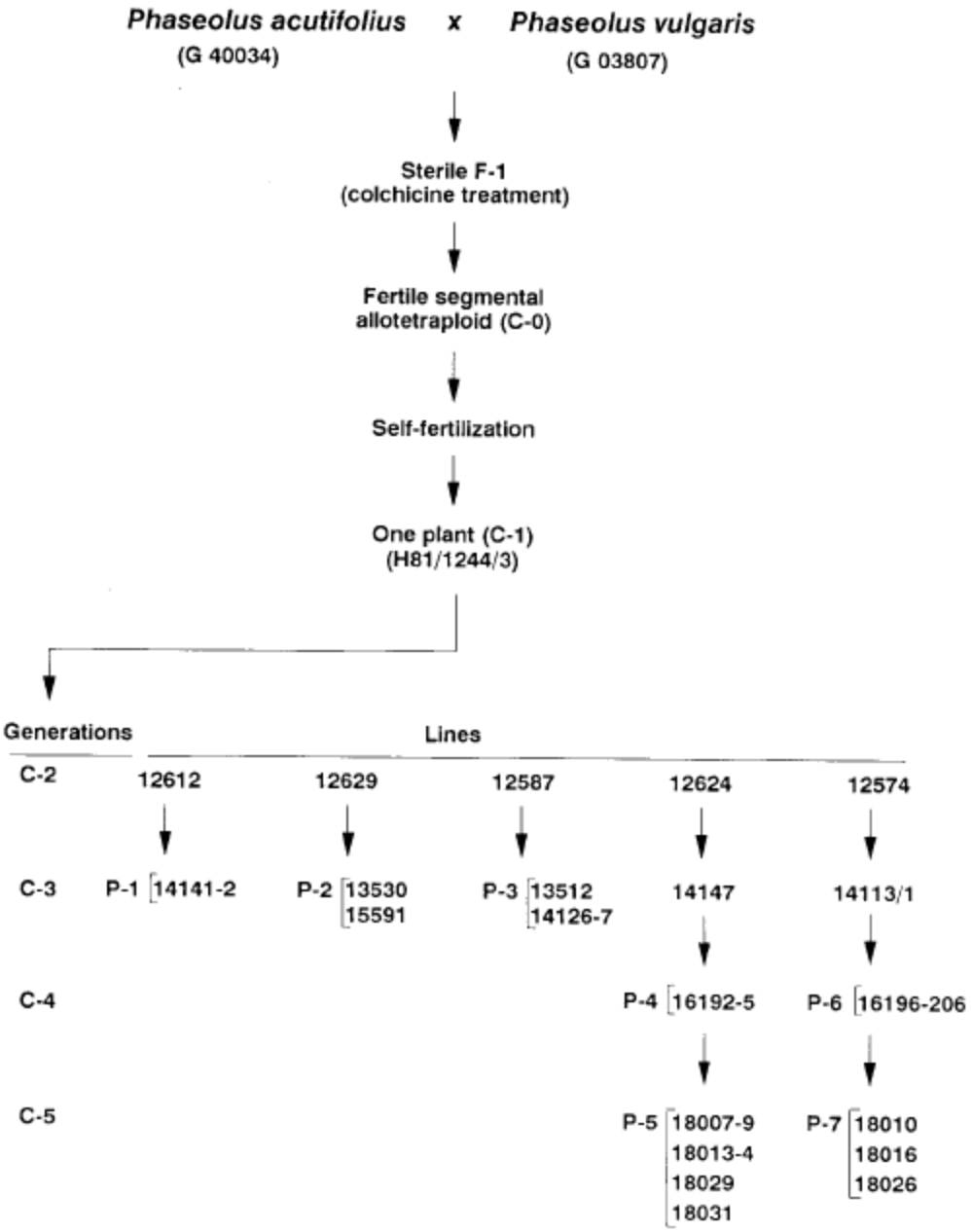

FIG. 1. Genealogy of *Phaseolus acutifolius* × *Phaseolus vulgaris* hybrids and their progeny.

Also, N_2 fixation in P. *vulgaris* has been extensively studied (Graham and Halliday 1976). In the case of *P. acutifolius,* however, besides its designation to the cowpea cross-inoculation group (Walker 1928; Carroll 1934), no research reports were found on investigations of its N_2-fixing potential. In the legume-rhizobial symbiosis, the genetic factors of the legume and the rhizobia influence the nodulation and N_2 fixation. Interspecific hybridization provides a means of transferring important disease resistance to *P. vulgaris.* In addition, it could provide a bridge for transferring the ability to fix N_Z with *Bradyrhizobium spp.*

The objective of this research was to examine more closely the cross-inoculation grouping of P. *acutifolius* and to assess the ability of interspecific hybrid progeny to nodulate and fix N_2 with R. l. bv. *phaseoli* and *Bradyrhizobium* isolates.

Materials and methods

Seeds

A white-seed cultivar of P. acutifolius was obtained from the NifTAL Project seed germ plasm collection. These seeds were used in experiment 1. In experiment 2, four seed types (brown, white, wild, and pinto) were provided by Dave Parsons, University of Arizona. The brown and white seeds were from P. acutifolius var. latifolius. Phaseolus vulgaris X P. acutifolius hybrid progeny seeds were used in experiment 3. The genealogy of these hybrid progeny is shown in Fig. 1. These seeds were allotetraploids developed at La Faculte des Sciences Agronomiques de 1'Etat a Gembloux, Belgium. The progeny of these allotetraploids were multiplied and observed during six generations to study the variability and the reaction to several diseases and pest in Colombia (L. Lewinson, personal communication). As a result of the low fertility of the hybrids in the early generations, all the progeny originated from the self-pollination of a single C_1

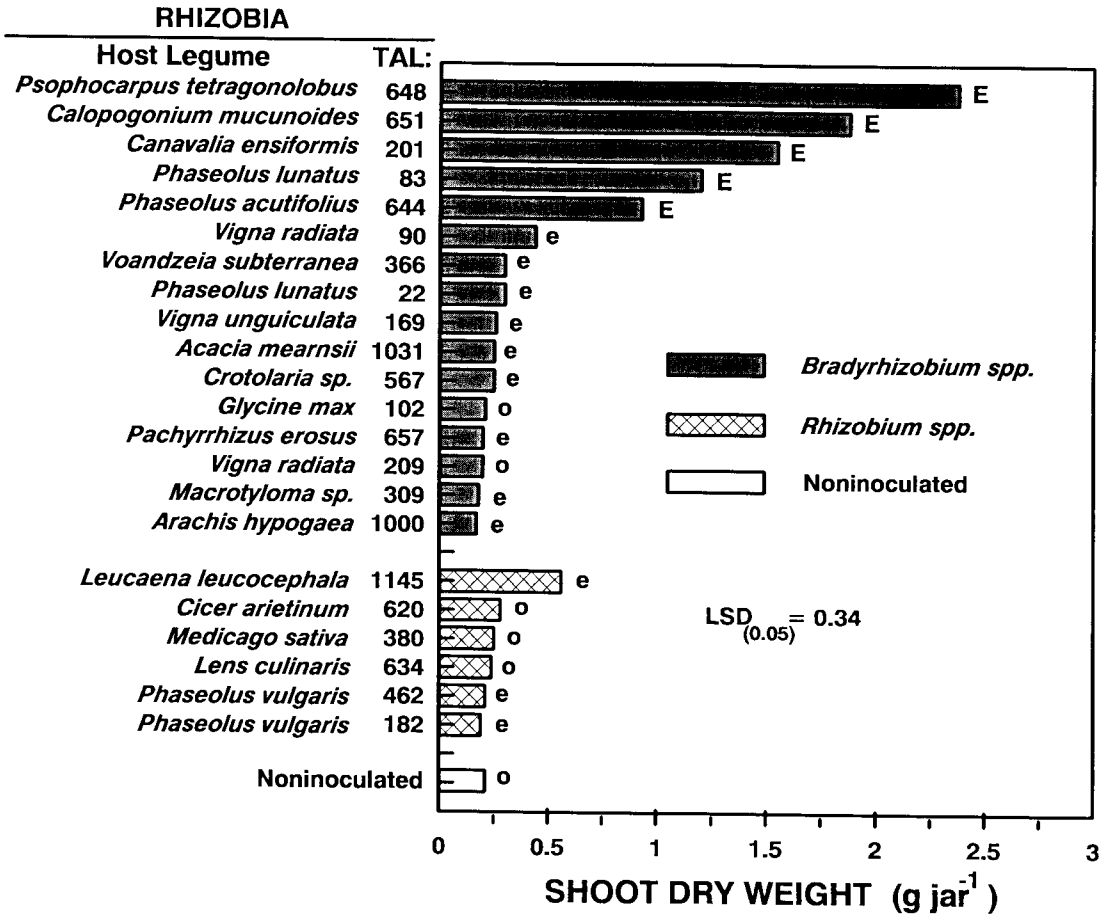

FIG. 2. Effectiveness of *Bradyrhizobium* spp. and *Rhizobium* spp. on *Phaseolus acutifolius*. E, effective nodulation; e, ineffective nodulation; o, no nodulation.

allotetraploid. The G_1 progeny was grown in a greenhouse for multiplication. This was followed by planting in open fields. A mixture of seeds was sampled from the multiplication field plots for studying the symbiotic potential of the progeny. The P. *acutifolius* parent (G40034) is a cultivar grown in the semi-arid zone of Lacatecoluca in El Salvador, while the P. *vulgaris* parent (G03807) is a traditional cultivar (Bico de Ouro) grown in the warm, low-altitude regions of Brazil. Seven lines (Fig. 1) of hybrid progeny from three generations (C_3, C_4, and C_5) were tested. For convenient reference, the seeds of the progeny are henceforth referred to by the prefix P and a number as indicated in Fig. 1.

Cultures

Rhizobial strains tested in experiments 1 and 3 were obtained from the University of Hawaii NifTAL Project Germplasm resource, and those investigated in experiment 2 were from the collection of the Nitragin Co., Milwaukee, WI. All strains were maintained on slants of yeast-mannitol (YM) agar (Vincent 1970) stored at 4°C.

Plant culture

In all experiments, undamaged seeds were selected for uniform size, surface sterilized in 3% (v/v) hydrogen peroxide for 5 min, rinsed in at least six changes of sterile water, and left to imbibe for 24 h in a refrigerator. Imbibed seeds were grown in moist, presterilized (autoclaved) vermiculite. Pregerminated seeds with straight radicles (1.0-1.5 cm) were sown and inoculated (Somasegaran and Hoben 1985) in modified Leonard jars (Vincent 1970) that used horticultural grade vermiculite or silica sand for the rooting medium. The N-free nutrient solution used in the Leonard jars was according to the formulation of Broughton and Dilworth (1971). Plants were grown in a greenhouse under natural illumination.

Experiment 1

Cross-inoculation relationships of P. *acutifolius* with rhizobia isolated from other legume hosts were investigated using 16 *Bradyrhizobium* strains and six *Rhizobium* strains (Fig. 2). Each treatment was set up in triplicate with two plants per replication. The experiment was a randomized complete block design. Uninoculated controls were included. Plants were harvested at 35 days. Nodulation observations were made after the root system was washed free of the rooting medium. The shoot was oven-dried at 70°C for 72 h.

Experiment 2

The extent of host X rhizobial strain interaction was studied in the brown, white, wild, and pinto genotypes of P. *acutifolius* in association with 11 strains of bradyrhizobia and a commercial ("EL" commercial mix) peat inoculant (Table 1). Owing to the limited availability of seeds, each treatment was set up in Leonard jars in duplicate. Ten seeds were sown per jar and inoculated with the appropriate culture. Plants were grown in a greenhouse and harvested after 30 (brown and white seeded genotypes) or 35 days (wild and pinto genotypes) after sowing. At harvest, observations were made to determine effective or ineffective nodulation based on the color and appearance of whole sliced nodules. Shoots were oven-dried and ground samples were processed for total N determination by the Kjeldahl method.

Experiment 3

The *(Brady)-Rhizobium* affinities and N,-fixing potential of the *P. acutifolius X P. vulgaris* hybrid progeny were tested by inoculation with two groups of rhizobia. Each group of rhizobia consisted of a mixture of three strains of either *R. leguminosarum* bv. *phaseoli*

TABLE 1. Symbiotic N_2 fixation in four *P. acutifolius* genotypes as influenced by isolates of bradyrhizobia[a]

Inocula	Original host	Brown seed		White seed		Wild		Pinto	
		Nodulation[b]	mg N[c]	Nodulation	mg N	Nodulation	mg N	Nodulation	mg N
Nitragin 32Z3	*Crotolaria* sp.	e	31(0)[d]	e	nd[e]	nd	nd	nd	nd
Nitragin 32H1	*C. paulina*	o	40(7)	e	27(0)	nd	nd	nd	nd
Nitragin 41Z10	*Desmodium* sp.	e	35(2)	e	30(0)	nd	nd	nd	nd
Nitragin 41F3	*D. uncinatum*	E	59(26)	e	nd	nd	nd	nd	nd
Nitragin 42B3	*Lablab purpureus*	e	34(1)	e	nd	nd	nd	nd	nd
Nitragin 127D3	*Vigna radiata*	o	38(5)	e	nd	E	67(53)	o	32(0)
Nitragin 127D6	*V. radiata*	nd	nd	nd	nd	o	13(0)	o	30(0)
Nitragin 176A22	*V. unguiculata*	e	33(0)	e	nd	o	16(0)	o	39(6)
Nitragin 127E12	*Phaseolus limensis*	E	64(31)	E	83(54)	E	51(37)	E	70(37)
Nitragin 127N2	*P. penduratus*	E	85(52)	E	85(56)	e	24(10)	E	77(44)
Nitragin 150B1	*Stylosanthes sundaica*	e	29(0)	e	29(0)	o	18(4)	o	27(0)
Nitragin EL commercial mix		E	63(30)	E	96(67)	nd	nd	nd	nd
Noninoculated		o	33	o	29	o	14	o	33

[a]Data of the late Dr. J. C. Burton.
[b]E, effective; e, ineffective; o, absence of nodules.
[c]Milligrams of nitrogen per 10 plants.
[d]Values in parentheses indicate mg N fixed (mg N of inoculated minus mg N of noninoculated).
[e]Not done.

(henceforth referred to as *R. l.* bv. *phaseoli)* or *Bradyrhizobium spp.* The R. l. bv. *phaseoli* mixture consisted of strains TAL 182, TAL 1383 (CIAT 632), and TAL 1797 (CIAT 899), while the *Bradyrhizobium* spp. mixture contained strains TAL 644 (CIAT 257), TAL 336 (USDA 3255), and TAL 648 (UMKL 57). The original host of isolation of TAL 644 and TAL 336 was P. *acutifolius,* but TAL 648 was originally isolated from the nodules of the winged bean *(Psophocarpus tetragonolobus).* All strains were cultured individually in yeast-mannitol broth to attain cell populations of approximately 2×10^9 cells mL^{-1}. Equal volumes of each strain were then mixed in a sterile Erlenmeyer flask to achieve a mixture of three strains of the appropriate group. Each jar was planted with four pregerminated seeds and inoculated with 1 mL of the mixed strain inoculum. The seven lines of the progeny and the two parents (see Fig. 1) were tested against the two groups of rhizobia. Five replications were set up for each treatment, including noninoculated controls. At 7 days, plants were thinned to two per jar. The experiment was a randomized complete block design and plants were harvested at 35 days. At harvest, nitrogenase activity was determined by the acetylene reduction assay as described previously (Somasegaran and Martin 1986). Shoot and nodule dry weights were determined after oven drying at 70°C for 72 h. N content of the shoot was determined as described by Mitchell (1972) using a Technicon autoanalyzer (Technicon Instruments, Tarrytown, NY).

Experiment 4

Two progeny (P-6 and P-7) and the P. *vulgaris* parent, which showed high N content in the shoot (experiment 3), were further tested for response to inoculation in potted soil. Peat-based inoculant containing equal numbers of R. l. bv. *phaseoli* strains TAL 182, TAL 1383, and TAL 1797 was prepared following the procedure previously described (Somasegaran and Hoben 1985). A tropical oxisol (clayey, ferritic, isohyperthermic, humoxic tropohumult; pH 4.8, Haiku series) with no native R. l. bv. *phaseoli* (Singleton and Tavares 1986) was used in the test. Soil preparation, liming, and nutrient additions are described elsewhere (Singleton *et al.* 1985). Seeds were surface sterilized and pregerminated. Four pregerminated seeds were planted per pot and inoculated (1.0 mL per seed) with a water-peat inoculant suspension (2×10^8 rhizobia mL^{-1}) of the three strains. There were inoculated and noninoculated controls for each seed type. All treatments were in quadruplicate and the experiment was a randomized complete block design. The experiment was terminated at 31 days. At harvest, shoot and nodule dry weights, nitrogenase activity, and total N content parameters were determined as described in experiment 3. Nodulation competitiveness of the inoculant strains was determined by the fluorescent antibody technique (Schmidt *et al.* 1968) using oven-dried root nodules (Somasegaran *et al.* 1983).

Results

The results showed that P. *acutifolius* has a highly specific bradyrhizobial requirement for effective nodulation (Fig. 2). Bradyrhizobial isolates TAL 648, TAL 651, TAL 201, TAL 83, and TAL 644 from the nodules of *Psophocarpus tetragonolobus, Calopogonium mucunoides, Canavalia ensiformis, Phaseolus lunatus,* and *P. acutifolius,* respectively, were effective, whereas most others caused ineffective nodulation or no nodulation. Among the *Rhizobium spp., R. l. bv. phaseoli* (TAL 182 and TAL 462) and *R. loti* (TAL 1145) *Leucaena leucocephala* caused ineffective nodulation.

Nodulation and N_2 fixation in four cultivars of *P. acutifolius* in response to inoculation with 11 strains of *Bradyrhizobium sp.* and an EL commercial mix (five bradyrhizobial strains) inoculant indicated strong host by *Bradyrhizobium* interaction (Table 1). Nodulation ranged from effective through ineffective to none. Only the *Bradyrhizobium* strain (Nitragin 127E12) isolated from *P. limensis* was highly effective on all four genotypes, whereas strain Nitragin 127N2 (P. *penduratus)* was ineffective on the wild genotype. Overall, of the 32 *Bradyrhizobium* X genotype combinations tested (excluding the EL commercial mix treatment), 72% (i.e., 28% with no nodulation and 44% with ineffective nodulation) did not result in N_2 fixation. Only 28% resulted in effective symbiosis and this was largely due to bradyrhizobia from *P. limensis* and *P. penduratus.*

The symbiotic potentials of the progeny of the P. *acutifolius* X *P. vulgaris* hybrids in response to inoculation with R. l. bv. *phaseoli* are shown in Table 2. All the progeny and the two parents were nodulated by *R. l.* bv. *phaseoli,* but the effectiveness varied. The progeny fell into two distinct groups based on the shoot total N. The first group consisted of progeny P-1, P-2, P-3, P-4, and P-5, which were ineffectively nodulated and did not show high N content in the shoot. The second group included progeny P-6 and P-7, which formed a highly effective symbiosis only when inoculated with R. l. bv. phaseoli. P-7 was

TABLE 2. Symbiotic variability in *P. vulgaris*, *P. acutifolius*, and their hybrid progeny inoculated with *Rhizobium leguminosarum* bv. *phaseoli*

	Parent species		*P. vulgaris* × *P. acutifolius* hybrid progeny						
	P. vulgaris	*P. acutifolius*	P-1	P-2	P-3	P-4	P-5	P-6	P-7
Shoot dry weight[a]	1375 (525)[d]	185 (270)	495 (510)	545 (575)	480 (520)	560 (560)	445 (495)	1285 (605)	1485 (625)
Nodule dry weight[a]	155	15	20	35	45	50	20	105	120
TNA	6.1	0	0.5	0.4	1.8	3.6	0.3	6.9	11.2
Shoot total N[c]	41.5 (3)[d]	1.4 (1.7)	4.3 (3.1)	3 (3.1)	5 (3)	8.4 (3)	2.9 (3)	37.1 (2.8)	43.8 (3.8)

[a]Milligrams per plant.
[b]Total nitrogenase activity (TNA) (micromoles C_2H_4 per plant per hour).
[c]Milligrams per plant.
[d]Data of noninoculated control. LSD ($p = 0.05$): shoot dry weight = 215; nodule dry weight = 20; TNA = 3.2; shoot total N = 3.6.

TABLE 3. Symbiotic variability in *P. vulgaris*, *P. acutifolius*, and their hybrid progeny inoculated with *Bradyrhizobium* sp.

	Parent species		*P. vulgaris* × *P. acutifolius* hybrid progeny						
	P. vulgaris	*P. acutifolius*	P-1	P-2	P-3	P-4	P-5	P-6	P-7
Shoot dry weight[a]	305 (525)[d]	285 (270)	435 (510)	605 (575)	865 (520)	635 (560)	625 (490)	400 (605)	495 (625)
Nodule dry weight[a]	65	30	55	80	120	80	90	95	110
TNA	0.2	1.5	3.4	5	7.2	5.1	5.4	0	0
Shoot total N[c]	2 (3)[d]	5.7 (1.7)	7.7 (3.1)	14.7 (3.1)	18.4 (2.9)	16.8 (2.9)	15.5 (3)	2.3 (2.8)	3.3 (3.8)

NOTE: Footnotes are as in Table 2. LSD ($p = 0.05$): shoot dry weight = 120; nodule dry weight = 25; TNA = 1.6; shoot total N = 3.3.

TABLE 4. Summary of significances of sources of variation in the analysis of variance for growth, nodulation, and N_2 fixation by *P. acutifolius* × *P. vulgaris* hybrid progeny inoculated with *R.l.* bv. *phaseoli* and *Bradyrhizobium* sp.

Source of variation	Dry weight		TNA	Shoot total N
	Shoot	Nodule		
Replication	ns	*	ns	ns
Rhizobia (R)	***	***	***	***
Progeny (P)	***	***	***	***
P × R	***	***	***	***

*, ***Indicate significance at $p = 0.05$ and $p < 0.001$, respectively.

significantly higher in shoot total N than P-6. The P. *vulgaris* did not differ significantly from P-7 in the shoot total N, although P-7 had a numerically higher value.

Inoculation of the seven progeny and the two parents with *Bradyrhizobium* strains showed essentially the same two groups as with R. l. bv. *phaseoli* but with directly opposite effectiveness responses (Table 3). Although P-6 and P-7 were nodulated by the bradyrhizobia, there was no nitrogenase activity detectable. These results indicated that the nodules, in spite of the high nodule dry weights, did not benefit the plant. Progeny P-1, P-2, P-3, P-4, and P-5 were effectively nodulated by *Bradyrhizobium* strains and showed a significantly higher level of shoot N than P-6 and (or) P-7 when the latter also were inoculated with *Bradyrhizobium* strains. Progeny P-1, P-2, P-3, P-4, and P-5 were of higher symbiotic potential than the P. *acutifolius* parent, as shown by the shoot total N of the progeny.

P-3 was the best progeny for N_2 fixation in symbiosis with the bradyrhizobia.

Analysis of variance, which excluded the data for the *P. acutifolius* and P. *vulgaris* parents and the noninoculated controls, showed that the main effects due to the progeny, the rhizobia, and the progeny X rhizobia interaction were highly significant ($p < 0.001$) for all the growth nodulation and N_z fixation parameters measured (Table 4). A more detailed analysis of variance was conducted on the shoot total N to quantify the main effects and the interaction. The analysis indicated that the progeny X rhizobia interaction accounted for 83% of the total phenotypic variation. Additive effects of rhizobia and progeny accounted for only 2.0 and 11.8%, respectively.

Progeny P-6 and P-7 showed higher symbiotic potential in Leonard jars than progeny P-1, P-2, P-3, P-4, and P-5, when inoculated with *R.* l. bv. *phaseoli,* so they were further tested for inoculation response in an oxisol (Table 5). The two progeny and the P. *vulgaris* parents showed significant increases in the total nitrogenase activity (TNA) and nodule dry weight as a result of inoculation with R. l. bv. *phaseoli* inoculants. However, significant differences in the shoot and total shoot N were not observed because of the high soil mineral N in the soil. Although nodules were observed in the noninoculated treatments of P-6, P-7, and P. *vulgaris,* the low TNA and nodule weights indicated that the nodulation was ineffective.

The most competitive of the three strains of R. l. bv. *phaseoli* was TAL 182 with TAL 1797 showing low competitiveness on P-6 and P. *vulgaris* (Table 6). In P-5, 16% of the nodules were not identifiable with the fluorescent antibodies of TAL 182, TAL 1383, or TAL 1797.

TABLE 5. Response of *P. acutifolius* × *P. vulgaris* hybrid progeny and *P. vulgaris* to inoculation with *R.l.* bv. *phaseoli* in an oxisol

Plant	Treatment[a]	TNA	Nodule dry weight (mg) per plant	Shoot dry weight (g) per plant	Shoot total N (mg) per plant
P-6	I	5.1	88	1.4	33.9
	N	0.1	2	1.1	27.3
P-7	I	5.2	114	1.4	33.8
	N	0.7	3	1.1	27.1
P. vulgaris	I	4.4	100	1.1	30.5
	N	0.3	3	0.9	19.4
$LSD_{(0.05)}$		1.4	40	0.3	11.5

[a]I, inoculated; N, noninoculated.

TABLE 6. Competition for nodulation by three strains of *R. leguminosarum* bv. *phaseoli* on *P. vulgaris*, and *P. acutifolius* × *P. vulgaris* hybrid progeny grown in an oxisol

	Nodule occupancy (% of total) by[a]:			
Plant	TAL 182	TAL 1383	TAL 1797	unidentified
P-5	84	0	0	16
P-6	84	0	16	0
P. vulgaris	98	0	2	0

[a]The three strains of *Rhizobium* were equally represented in the peat-based mixed inoculum. Fifteen nodules were typed from each replication.

Discussion

The earliest reports that indicated the cross-inoculation group of P. acutifolius were by Walker (1928) and Carroll (1934) in which this legume is listed in the cowpea cross-inoculation group. After this period no further information was found reporting on the nodulation characteristics and N_z fixing potential of P. acutifolius. The promiscuity for nodulation by P. acutifolius resembled the properties of P. vulgaris, which nodulated with a wide range of bradyrhizobia, but N_z fixation only occurred when nodulation was caused by R. l. bv. phaseoli (Lange 1961).

Strain selection constitutes a necessary first step to develop effective inoculant rhizobia for a relatively little studied legume such as P. acutifolius, which has attracted much recent interest because of its importance in breeding experiments involving P. vulgaris. When there is an initial indication of specificity in the rhizobial requirement for N_Z fixation (Fig. 2), it becomes necessary to investigate the occurrence of specificity in other wild and cultivated genotypes of P. acutifolius.

Considerable specificity for effective N_z fixation was demonstrated in the four host genotypes tested against the strains of bradyrhizobia (Table 1). Generally, bradyrhizobia that were isolated from P. lunatus, P. limensis, P. penduratus, P. acutifolius, Canavalia ensifonnis, Calopogonium mucunoides, and Psophocarpus tetragonologus appeared more likely to cause effective symbiosis on P. acutifolius than other bradyrhizobia (Fig. 2 and Table 1). These observations need to be tested further in strain selection programs for P. acutifolius.

Improvement in the symbiotic potential over the parent genotypes was most noticeable in the progeny that were effectively nodulated by the bradyrhizobia (Table 3). This was illustrated especially by P-2, P-3, P-4, and P-5. In the case of P-3, which appeared to be the best macrosymbiont, its total shoot N was 222.8% greater than that of the P. acutifolius parent. This is in contrast to results observed for P-6 and P-7, which fixed N_Z effectively with R. l. bv. phaseoli but with no dramatic increases in shoot N.

Hybridization of P. acutifolius with P. vulgaris resulted in two groups of hybrid progeny that were able to form nodules with two distinct species of rhizobia. In the classical approach, assignment of a legume to a particular cross-inoculation group is based on cross-inoculation studies between the various species of rhizobia and the legume in question. In a specified cross-inoculation group, the rhizobia from the various legumes in that group are mutually interchangeable (Fred et al. 1932). In the case of the progeny, there was interchangeability for the formation of nodules (infectiveness) but not for effectiveness (N_Z fixation). Based on the infectiveness properties, the progeny may be placed in either the bean or cowpea cross-inoculation groups. However, considering practical reasons under which effectiveness is the important criterion, progeny P-6 and P-7 would be classified in the bean group while P-1, P-2, P-3, P-4, and P-5 would be assigned to the cowpea group.

It is important to emphasize that the division of the progeny into two distinct cross-inoculation groups is based on a very narrow sample of plant genotypes. In this narrow sample none of the progeny exhibited effective symbiosis with both species of rhizobia tested. However, it is important to note that there is evidence of the occurrence of possibly a small number of genes, determining with which rhizobia the hybrid progeny will fix N_2 effectively. Further studies with a larger number of plant genotypes will be required to determine the inheritance of the N_2 fixation pattern observed in this work.

By inspection of the data in Tables 2 and 3, it was clear that the rhizobia *x* progeny interaction represented the largest effect, accounting for 83% of the variability. This was expected because the progeny fixed N_2 either with R. l. bv. phaseoli or with Bradyrhizobium strains. In an experiment involving 10 strains of R. l. bv. phaseoli and three cultivars of P. vulgaris, no significant host *x* strain interaction was observed, indicating no specific combination was superior over the broad range of all treatments (Pacovsky et al. 1984).

The oxisol used to test the inoculation responses of P-6 and P-7 has been shown to have native bradyrhizobial populations that nodulate Arachis hypogaea, P. lunatus, and Vigna unguiculata (Singleton and Tavares 1986). However, the native bradyrhizobia competed very little for nodulation as 84% of the nodules on P-6 and P-7 were formed by R. l. bv. phaseoli strain TAL 182 (Table 6). The soil test further validates the observation that P-6 and P-7 belong to the bean cross inoculation group and confirms the identification of an effective inoculant for use on the two progeny.

It is clear from this investigation that nodulation studies on P. acutifolius *x* P. vulgaris hybrid progeny need to be instituted as an essential part of the program on P. vulgaris germplasm improvement. Nodulation studies will ensure recognition of the rhizobial requirements of the progeny and hence lead to the proper utilization of their symbiotic potential.

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