Rhizobium inoculation of *Phaseolus vulgaris* in Lajas, Puerto Rico[1]

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ABSTRACT

A field experiment was conducted to evaluate the effect of *Rhizobium* inoculation methods and the effect of different levels of N fertilization on nodulation, nitrogen fixation and yield of *Phaseolus vulgaris* L. The N treatment produced a seed yield significantly higher than that of the other treatments. Inoculation treatments had no significant effect on nodule number and dry weight, even when compared to the noninoculated and N controls. This finding was attributed to a high number of *Rhizobium phaseoli* in the soil. The applications of N and inoculation did not significantly affect the N percentages of foliage and grain or nitrogenase activity. Serological identification of nodules indicated that the native strain UPRM 6000 formed the largest percentage of nodules (41%), followed by *R. phaseoli* 127K44 (34%), 127K17 (13%) and 127K12b (10%). The serogroup distributions of inoculated and N fertilized treatments differed significantly from the noninoculated control. Inoculation methods (seed vs. soil) did not differ significantly in either nodulation or yield responses.

RESUMEN

Inoculación de *Phaseolus vulgaris* con *Rhizobium* en Lajas, Puerto Rico

Se hizo un experimento de campo para estudiar el efecto de métodos de inocular *Rhizobium* y el efecto de diferentes cantidades de abono nitrogenado sobre la nodulación, fijación de nitrógeno y rendimiento de *Phaseolus vulgaris* L. El tratamiento control con N produjo un rendimiento de semilla significativamente superior al de los otros tratamientos. Los tratamientos de inoculación no tuvieron efecto significativo sobre el número y el peso seco de los nódulos, aun comparándolos con los de los testigos no inoculados y con N. Esto se atribuyó a un alto número de *Rhizobium phaseoli* en el suelo. Las aplicaciones de N y la inoculación no aumentaron significativamente los porcentajes de N en el follaje ni en el grano; tampoco aumentaron la actividad nitrogenásica.

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La identificación serológica de los nódulos indicó que la cepa nativa UPRM 6000 formó el mayor porcentaje de nódulos (41%), seguida por *R. phaseoli* 127K44 (34%), 127K17 (13%) y 127K12b (10%). La distribución en serogrupos de los tratamientos inoculados y abonados con N fue significativamente diferente del testigo no inoculado. Los métodos de inoculación (semilla contra suelo) no presentaron diferencias significativas ni en nodulación ni en rendimiento.

INTRODUCTION

Dry beans (*Phaseolus vulgaris* L.) are one of the world's most important legumes grown for human nutrition. Inoculation with superior strains of *Rhizobium phaseoli* is a practice intended to improve low bean yields, especially in developing countries (1).

Bean response to inoculation under field conditions has been extremely variable, and results up to 1977 have been reviewed (7). More recently, a study in eastern Africa (11) showed yield responses in spite of the presence of indigenous rhizobia. Research in England (21) reported maximum nodulation, nitrogen fixation, and plant growth with inoculation. In El Salvador, inoculant effects on yield and nodulation were dependent on plant growth and location (17).

Nitrogen fertilization of beans has produced variable effects on symbiosis. Inhibition of nodulation has been observed when nitrate concentration is greater than 27 p/m (14) whereas optimum nodulation and yields have been produced with inoculation and 40 kg/ha of applied N (12). Modest "starter" amounts are considered to promote nodulation (7). Field trials with supplemental N have demonstrated that the N derived from N fixation can be, but is not always, a limiting factor to maximum yields (6,10,19).

The method of inoculation can be an important factor. In Honduras, the granular inoculant form produced significantly higher yields than the pellet form (2). Yields from inoculated treatments were similar to those obtained with 60 or 200 kg N/ha.

The objective of this experiment was to evaluate the inoculation effect in bean responses to nodulation, N fixation, recovery of inoculant strains, and yields. In order to assess factors affecting this practice, treatments of applied N and different inoculation methods were included.

MATERIALS AND METHODS

The experiment was conducted in field plots at the Lajas Research and Development Center of the University of Puerto Rico. The soil was a Fraternidad series. (Udic Chromusterts) with the following laboratory analysis (0 to 20 cm soil depth): pH 6.4; 10 p/m P; 231 p/m K; 3344 p/m Ca; 619 p/m Mg; and 2.97% organic matter. The background population of *Rhizobium* was 1.7×10^3/g dry soil, as determined by the most probable number technique (MPN) (22). Four-row plots 6.1 m long were used with 0.6 m between rows.

Seeds of cultivar "Arroyo Loro No. 1" were planted at a population density of 167,000/ha at a seed spacing of 0.1 m. At 39 (V5 growth stage) and 56 (R4 growth stage) days after planting (DAP) measurements of nodule number and dry weight, plant top dry weight, acetylene reduction, and % N were taken from 5 plants at the end of a center row in each plot. Nodules were dried at 90° C for 24 hours, and plants were dried until a constant dry weight was obtained. The % N was determined by the micro-Kjeldahl method with a Tecator model 1002 Kjeltec System[3]. Acetylene reduction activity for 1 hour was recorded by a gas chromatograph, with a Perking-Elmer model Sigma 4B with a Poropak N column at 60° C. Serological identification of nodules was done by the micro-agglutination technique (22) with prepared antisera for strains 127K44, 127K12b, 127K17, and UPRM 6000. At 78 DAP, the 2 center rows were harvested for seed yield, % protein, and total protein. For the duration of the experiment (December to March), all plots received sprinkler irrigation and manual weeding.

The experimental treatments consisted of a 2 × 2 factorial combination of N fertilization and inoculation method. The two levels of N applied at planting as ammonium sulfate were 0 and 20 kg N/ha The inoculation methods applied at planting were powder (rate: 150 g/45.5 kg of seed) and granular (rate: 56 kg/ha peat, and each provided 18×10^6 rhizobia/g of inoculant, as determined by MPN. The inoculants were supplied by Nitragin Sales Corp. and contained strains 127K44 and 127K12b. The experiment included a control treatment which received neither inoculation nor N fertilizer, and a N+ treatment which received 100 kg N/ha as ammonium sulfate at planting and no inoculation. All plots received 100 kg P/ha as triple superphosphate.

The six treatments were arranged in a randomized complete block design with four replications. The measured variables were statistically analyzed in an ANOVA. The means of the treatments were compared with the Duncan's Multiple Range Test at the 5% probability level of significance. In addition, an ANOVA, without the data of the N+ treatment, was performed to compare orthogonal contrasts of N fertilization and inoculation. The results of serology were analyzed by a chi-square test for count data at the 5% probability level of significance; the strain distribution of the control treatment was the expected outcome. Correlations were calculated for all the measured variables.

[3]Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

TABLE 1.—*Orthogonal contrasts for inoculation types (I) and N fertilizer (N) and the* F *test for nodulation, acetylene reduction activity (ARA) and plant growth at two growth stages (V5 and R4), and seed yield of* Phaseolus vulgaris *L.*

	Nodule weight		Nodule number		Weight		Plant top %N		Protein		Total ARA		Specific ARA		Seed yield
Component[1]	V5	R4	V5	R4	V5	R4	V5	R4	V5	R4	V5	R4	V5	R4	
	significance[2]														
I	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Ip vs Ig	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
NO vs N_2O	NS	NS	NS	NS	**	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
I × N	NS	**	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
B	NS	*	NS	NS	**	NS	**	NS	**	NS	NS	NS	NS	NS	**

[1]I = Response to inoculation; Ip vs Ig = Response to powder compared to granular inoculation; No vs N_2O = Response to 0 compared to 20 kg N ha-1; I vs N = Response to inoculation method × N fertilizer interaction.

[2]*, ** = Significant at the 0.05 and 0.01 levels, respectively; NS = Non-significant at the 0.05 level.

RESULTS AND DISCUSSION

There was an inoculation response of nodule number at R4 compared to the control, and the interaction effect was significant for both nodule number at R4 (table 1). The greatest nodule number at R4 was produced by peat inoculation and granular inoculation with 20 kg N/ha. Positive correlation between nodule weight and number were significant only at R4 (table 2).

The observed nodulation of the control treatment, a reflection of the background population of rhizobia, fell within the required range of 10^3 to 10^6 rhizobia/g for abundant nodulation (22). Therefore, it is noteworthy that a significant inoculation response of nodule number at R4 was observed even with a high rhizobial population. The lack of nodulation inhibition by the N+ treatment did not agree with a previous study (16), which observed inhibition with 50 kg N/ha. The lack of a "starter" N response on nodulation also did not agree with previous results (7). Nodulation differences between inoculation methods were not obtained which differed with a study of soybeans (18) in Puerto Rico, where granular inoculation significantly increased nodulation compared to powder and liquid inoculations. Mean nodule number averaged over treatments (table 3) decreased 30% from V5 to R4 which differed with a study (9), where maximum nodule number was found at 56 and 63 DAP, and agreed with a report (5) of maximum nodule number at 21 days after emergence.

TABLE 2.–*Correlation coefficients among observed parameters at two growth stages (V5 and R4) and harvest in* Phaseolus vulgaris *L.*

Parameters[1]	Correlation[2]		
	V5	R4	Harvest
Nodule number and nodule weight	0.629	0.981**	
Nodule number and plant top weight	0.636	–0.754**	
Nodule number and total ARA	0.529	0.910*	
Nodule number and specific ARA	–0.046	0.309	
Nodule number and seed yield	0.834*	–0.202	
Nodule weight and plant top weight	0.680	–0.689	
Nodule weight and total ARA	0.939**	0.838*	
Nodule weight and specific ARA	0.699	0.183	
Nodule weight and seed yield	0.819*	–0.151	
Plant top weight and plant % protein	0.990**	0.993**	
Plant top weight and seed yield	0.751	0.610	
Total ARA and specific ARA	0.793	0.595	
Total ARA and seed yield	0.853*	0.019	
Specific ARA and seed yield	0.363	0.278	
Seed yield and seed % protein			0.212
Seed yield and total seed protein			0.981**

[1] ARA = Acetylene reduction activity.

[2] *, ** = Significant at 0.05 and 0.01 levels, respectively (Pearson's r' correlations).

TABLE 3.—*Effect of inoculation (I) and nitrogen (N) on* Phaseolus vulgaris *L. nodulation at two growth stages (V5 and R4)*

Treatment	Nodule number		Nodule dry weight	
	V5	R4	V5	R4
	Number/plant		*mg/plant*	
Ip and N_2O	29.60 a[2]	9.50 b	17.26 a	9.78 a
Ip and NO	25.25 a	30.45 a	20.32 a	25.67 a
Ig and N_2O	21.90 a	29.15 a	14.18 a	26.89 a
Ig and NO	29.85 a	18.10 b	12.77 a	18.19 a
Control	28.80 a	14.85 b	22.78 a	19.49 a
N+	37.95 a	18.65 b	26.43 a	19.49 a
Mean	28.89	20.12	18.96	18.95

[1]Ip, Ig = Powder and granular inoculations, respectively; NO, N_2O = 0 and 20 kg N/ha, respectively; Control = No inoculation and no N fertilizer; N+ = 100 kg N/ha, and no inoculation.

[2]Means followed by one or more letters in common do not differ significantly at the 5% level by Duncan's multiple range test.

N+ treatment produced greater plant top weight and total protein at V5 (table 4) In addition, the orthogonal class contrasts (table 1) indicated a significant "starter" response of plant top weight and total protein at V5. These results demonstrated that N derived from N fixation was a limiting factor to plant growth at V5 and the need for supplemental N. Similar treatment responses of plant top weight and total protein reflected their positive correlation at both V5 and R4 (table 2). The lack of significant effects on % N demonstrated that neither inoculation nor N fertilization affects the % N of dry beans.

TABLE 4.—*Effect of inoculation (I) and nitrogen fertilization (N) on plant top dry weight, % N and total protein at two growth stages (V5 and R4) in* Phaseolus vulgaris *L.*

Treatment[1]	Top dry weight		Nitrogen		Total protein	
	V5	R4	V5	R4	V5	R4
	g/plant		*%*		*mg/plant*	
Ip and N_2O	2.32 b[2]	6.88 a	2.46 a	2.41 a	373.00 b	1039.25 a
Ip and NO	1.69 b	3.25 a	2.61 a	2.56 a	293.50 b	519.50 a
Ig and N_2O	2.19 b	3.02 a	2.22 a	2.48 a	352.50 b	475.50 a
Ig and NO	1.36 b	4.41 a	2.42 a	2.56 a	212.50 b	704.25 a
Control	1.98 b	3.67 a	2.51 a	2.60 a	325.25 b	604.50 a
N+	3.38 a	6.15 a	2.91 a	2.32 a	624.75 a	884.25 a
Mean	2.15	4.56	2.52	2.49	363.58	704.54

[1]Ip, Ig = Powder and granular inoculations, respectively; NO, N_2O = 0 and 20 kg N/ha, respectively; Control = No inoculation and no N fertilizer; N+ = 100 kg N/ha, and no inoculation.

[2]Means followed by one or more letters in common do not differ significantly at the 5% level by Duncan's multiple range test.

Bean nitrogen fixation was measured by the rates of total (per plant) and specific (per g nodule) acetylene reduction. Table 1 shows the lack of significant effects of inoculation or N fertilization on N fixation at both V5 and R4. A significant positive correlation between nodule weight and total acetylene reduction was found for both V5 and R4, in agreement with previous results (13). The mean rate of total acetylene reduction averaged over treatments was greater at V5 than at R4 (420 vs. 56 nmoles/plant/hr) which agreed with two previous studies (20,21) that obtained maximum activity at flowering initiation or 56 DAP. Specific activity also decreased from V5 to R4 (14.66 vs. 1.43 nmoles/g/h), in agreement with a previous study (8), and was attributed to the reallocation of the photosynthate supply in the bean plant from nodule to the pod sink during reproduction.

The N+ treatment produced a greater seed yield and total protein than the other treatments (table 5). The positive N response demonstrated that N fixation did not provide sufficient N for maximum yields, in agreement with other studies which obtained maximum seed production with high supplemental N (3,4). There was a significant correlation between nodule weight and seed yield at V5 (table 2). Inoculation and N fertilization did not affect the protein content of beans. Previous results (15) reported no relationship between an observed positive inoculation response of nodulation and the final seed % N. The two granular inoculation treatments produced yields similar to the two powder inoculation treatments.

Nodule recovery of *R. phaseoli* strains differed for the experimental treatments. At both V5 and R4 all of the inoculation treatments, except granular inoculation without N at V5, produced greater recovery of the inoculant strains (table 6). The presence of inoculant strains in the control

TABLE 5.—*Effect of inoculation (I) and nitrogen fertilization (N) on yield, protein percentage and total protein in* Phaseolus vulgaris *L. seeds*

Treatment[1]	Yield	Protein	Total protein
	kg/ha	*%*	*kg/ha*
Ip and N_2O	1461 b[2]	21.18 a	314 b
Ip and NO	1493 b	22.11 a	332 b
Ig and N_2O	1164 b	23.26 a	274 b
Ig and NO	1280 b	21.29 a	272 b
Control	1351 b	21.67 a	298 b
N+	1863 a	23.04 a	425 a
Mean	1435	22.09	320

[1] Ip, Ig = Peat and granular inoculations, respectively; NO, N_2O = 0 and 20 kg N/ha, respectively; Control = No inoculation and no N fertilizer; N+ = 100 kg N/ha, and no inoculation.

[2] Means followed by one or more letters in common do not differ significantly at the 5% level by Duncan's multiple range test.

TABLE 6.—*Effect of inoculation (I) and nitrogen fertilization (N) on the frequency distribution of serologically identified* Rhizobium phaseoli *strains in* Phaseolus vulgaris *L. at two growth stages (V5 and R4)*

Treatment[1]	127K44[2]		127K17		127K12b[2]		UPRM 6000		Others		P[3]	
	V5	R4	V5	R4	V5	R4	V5	R4	V5	R4	V5	R4
	%											
Ip and N_2O	45.0	36.2	6.7	34.0	6.7	4.3	36.7	23.4	5.0	2.1	*	*
Ip and NO	41.8	36.8	14.5	29.4	16.4	2.9	23.6	29.4	3.6	1.5	*	*
Ig and N_2O	54.2	23.0	12.5	41.0	10.4	13.1	22.9	21.3	0	1.6	*	*
Ig and NO	28.3	29.4	19.6	33.3	2.2	5.9	47.8	31.4	2.2	0	NS	*
Control	20.6	27.6	17.6	21.1	2.9	1.0	52.1	48.5	5.9	2.6		
N+	22.4	21.9	9.2	42.2	17.1	9.4	51.3	25.0	0	1.6	*	*
Mean	34.0	28.9	13.0	33.0	9.6	5.7	40.5	30.8	2.8	1.6		

[1]Ip, Ig = Powder and granular inoculations, respectively; NO, N_2O = 0 and 20 kg N/ha, respectively; Control = No inoculation and no N fertilizer; N+ = 100 kg N/ha, and no inoculation.

[2]Strains included in the inoculants.

[3]P = Probability of a significant difference in *chi*-square distribution against the control distribution; * = Significant at 5% level; NS = Non-significant at 5% level.

treatment indicated that these strains were introduced in previous bean plantings or that some of the native strains reacted with the testing antisera. The combined results of a significant inoculation response of reduced nodulation by native strains (e.g., UPRM 6000) compared to the control, and a lack of significant inoculation responses of yield and N fixation compared to the control (table 1) indicated that inoculant and native strains were equally effective, and highlighted the need to consider the role of the host genotype on N fixation.

The nodulation distributions indicated that the native strains formed the largest percentage of nodules, whereas 127K44 was the most competitive of the inoculant strains. In order to obtain further inoculation benefits, it is recommended that more competitive and effective *R. phaseoli* strains be selected for future experiments. Studies are underway to evaluate the potential of different bean cultivars for N fixation.

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