# Response of Bean Varieties to Inoculation with Selected *Rhizobium* Strains in El Salvador[1]

*P. Pineda*, J. Kipe-Nolt***

ABSTRACT

Farm surveys in two important bean-growing regions of El Salvador indicated very poor nodulation of local *Phaseolus vulgaris* varieties with the native *Rhizobium* populations. Fifty *Rhizobium* strains were isolated and evaluated for their ability to stimulate plant growth in the presence of native rizobia. Seventeen strains were evaluated in the glasshouse in two soils using the bean variety Rojo de Seda. Six of these strains were selected for a field trial. Inoculation increased nodulation but yield differences were not significant (P = 0,05) in this experiment. Twenty-eight strains were screened with 'Rojo de Seda' and 'RAB-204' in a second glasshouse trial. From this group, four strains were selected for field testing. Both nodulation and yield were increased significantly as a result of inoculation. Yield increments up to 945 kg/ha were obtained. Four promising bean varieties and the local commercial variety were evaluated for their response to nitrogen fertilizer and *Rhizobium* inoculation in a third field trial. Inoculation resulted in a significant increase in yield from 1 654 to 2 224 kg/ha, similar to the increase resulting from 180 kg urea/ha (2 477 kg/ha). Differences between genotypes in ability to nodulate and fix nitrogen (yield under low N conditions) were observed but require further confirmation.

COMPENDIO

Se efectuó un reconocimiento en dos zonas frijoleras de El Salvador que indició muy pobre nodulación en las variedades locales de *Phaseolus vulgaris* con las poblaciones nativas de *rhizobia*. Se aislaron 50 cepas de *rhizobia* y se evaluaron en invernadero, por su habilidad para inducir un aumento en el crecimiento de la planta en la presencia de *rhizobia* nativos. Se evaluaron 17 cepas en dos suelos con la variedad local, Rojo de Seda. De las cuales se seleccionaron seis para un ensayo de campo. La inoculación en el campo aumentó la nodulación, pero las diferencias en rendimiento no fueron significativas al 5%. En otro ensayo de invernadero, se emplearon 28 cepas con dos genotipos de frijol, Rojo de Seda y RAB-204. De este grupo se seleccionaron cuatro cepas para un ensayo de campo. Nuevamente la nodulación aumentó con la inoculación y en ese ensayo se observaron incrementos en el rendimiento hasta 945 kg/ha. Cuatro líneas promisorias de frijol y la variedad local comercial fueron evaluadas por su respuesta a la fertilización con nitrógeno y a la inoculación, en un tercer ensayo de campo. La inoculación resultó en un aumento significativo en el rendimiento de 1 654 a 2 224 kg/ha similar al aumento resultante de la aplicación de 180 kg urea/ha (2 477 kg/ha). Se notaron diferencias entre genotipos con respecto a su habilidad para nodular y fijar nitrógeno (rendimiento en condiciones de bajo nitrógeno) pero requiere de mayor confirmación.

## INTRODUCTION

Bean (*Phaseolus vulgaris* L.) is the most important legume and source of protein for human consumption in El Salvador. In spite of this it is primarily a subsistence crop cultivated by small farmers with suboptimal agronomic management, resulting in mean yields of 500 kg/ha (MAG, El Salvador, 1984). Approximately 60 000 ha of beans are planted annually, part in monoculture and part in association with maize.

Fertilizers are costly and often not available to the small farmer, so alternatives for overcoming nutrient constraints in beans need to be investigated. One alternative is to improve the nitrogen-fixing symbiosis of this legume with the bacterium *Rhizobium leguminosarum* biovar *phaseoli*. Genetic diversity for both components of the symbiosis has been demonstrated and offers potential for improvement. The success of manipulating the *Rhizobium* component through inoculation has been variable (2, 5, 8) often because of the lack of competitive ability of the inoculant strain in local soil conditions. Several plant breeding programs are working to increase the fixation potential of commercially acceptable bean genotypes (1, 6, 9) but as yet no materials from these programs are being grown at a commercial level. Preliminary studies indicated variability in the effectiveness of nodulation of beans with native *Rhizobium* populations and some potential for inoculation in El Salvador (11), however, inoculants have never been used commercially. No evaluation of the fixation potential of the local varieties has been carried out.

The objectives of this study were to evaluate nodulation with native *rhizobia* in farmers' fields; to selected competitive and more effective strains; to

1 Received for publication 25 January 1989

* Universidad de El Salvador, AA747, San Salvador, El Salvador. Present address: CIAT-IICA Apartado 14-0185 Lima 14, Perú

** Centro Internacional de Agricultura Tropical (CIAT), AA6713, Cali, Colombia

test these in yield inoculation experiments; and also to evaluate commercial and newly introduced bean germplasm for potential to nodulate and fix nitrogen. The goal is to increase nitrogen fixation by improving the *Rhizobium* strain and/or plant genotype component of the symbiosis.

## MATERIALS AND METHODS

### *Rhizobium* isolation

More than 100 farms in two important bean-growing regions of El Salvador were visited: Ahuachapán and Santa Ana in the eastern region, and Cabalsa, Cuscatlán and San Vicente in the central region. Plants were dug up carefully and nodulation evaluated. Large, firm, red nodules were collected for *Rhizobium* isolation. The bacteria were isolated and purified on yeast manitol agar (YMA). *R. phaseoli*-like colonies were further evaluated on YMA with bromothymol blue pH indicator, gram stained, tested for the presence of ketolactase and streaked on peptone-glucose medium to confirm purity (10, 13).

### Strain screening

Strain-screening trials were conducted in the glasshouse of the Agronomic Science Faculty of the University of El Salvador. The first experiments were planted in July of 1986 and the final one in January of 1987. Two-kg clay pots were used and one kg of soil was mixed with one kg of pumice stone to facilitate good drainage. Treatments were applied in a randomized complete block design with four repetitions.

Twelve *Rhizobium* isolates from the eastern zone (coded I for Ilobasco) were evaluated in a vertisol from that region. At the same time, 12 strains from the central zone (coded F for Frontera) were tested in an ultisol collected in Frontera. Five check strains from CIAT, a plus N treatment (equivalent to 100 kg N/ha in the form of ammonium nitrate) and a noninoculated control were included in both soils. Four seeds of the farmers' variety, Rojo de Seda, were planted in each pot and inoculated with 5 ml of culture medium containing approximately $1 \times 10^8$ cells/ml. After germination the plants were thinned to two per pot.

In a third trial, 25 local isolates and three check strains from CIAT were evaluated in an ultisol from Candelaria de la Frontera using the bean varieties Rojo de Seda and RAB-204. Noninoculated and nitrogen fertilized controls were included.

Thirty-five days after germination the plants were harvested and processed for nodule number, nodule dry weight, shoot dry weight and percentage N (using micro-Kkeldahl). The shoot nitrogen content was then calculated.

### Field inoculation trials

Selected strains were evaluated in three field trials in the cooperative San Antonio Zacamil in Candelaria de la Frontera in the department of Santa Ana. The soil was an ultisol of ph 5.2 with low P (3 ppm Bray II) and adequate K (138 ppm). The altitude was 700 m above sea level, mean annual rainfall, 1 485 mm, and temperature 23.5°C.

The effect of inoculating the variety Rojo de Seda with strains I-2, I-8, I-9, I-10, CIAT-632 and CIAT-899 was evaluated between September and November 1986. Granular inoculants were prepared in sterile peat obtained from the University of Costa Rica. They were applied to the soil at a rate of 2 g/m row, at the time of sowing. Two control, non inoculated, treatments were also included; one with nitrogen fertilizer (equivalent to 100 kg urea/ha applied at the time of planting), and the other without nitrogen. A randomized complete block design with three repetitions was used. Each plot consisted of rows, 4 m long with 0.20 m between plants and 0.50 m between rows. Two alley rows were planted between plots to help avoid contamination. Twenty days after germination 12 plants were dug and the nodules counted; the percentage of effective-appearing nodules (red internal color) was also estimated. At 40 days after germination six plants were dug up to evaluate nodulation (number of red nodules) and the shoots were dried and weighed as a measure of plant vigor. Grain yield of the central three rows of the plot was determined at maturity.

The effects of inoculating the bean variety RAB-204 with five *Rhizobium* strains, R-3, J-12, A-15, P-22 and I-10, were evaluated between May and July 1987. Noninoculated control treatments were included; the equivalent of 180 kg urea/ha (split into three applications) was used for the +N treatment. The inoculation method and experimental design were as in the first trial, but each plot four, 3 m long rows.

During the same season, five different bean genotypes were evaluated under three nitrogen treatments in a factorial experiment. The bean genotypes were: Rojo de Sede, Centa-Izalco, RAB-204, RAB-310 and RAB-404; and the nitrogen treatments were, 1) noninoculated and nonfertilized; 2) inoculated with a mixed-strain inoculant R-3, J-12 and P-22); and 3) nitrogen fertilized (3 applications equivalent to a total of 180 kg urea/ha). A split plot design was used, with bean genotypes as the main plots and nitrogen treatments as the subplots. There were four repetitions and each plot had four, 3 m rows. The equivalent of 60 kg P/ha was applied to the entire experiment and Benomyl, Parathion and Metamidophos were used to control pests.

Nodulation was evaluated at 20 days (12 plants/sample) and 50 days (six plants/sample) after germination. Shoot dry weight at 50 days, and grain yield at maturity, were also determined.

## RESULTS AND DISCUSSION

*Rhizobium* isolation

Approximately 100 farms were visited and plants in the mid-flowering stage were evaluated for nodulation with the native *Rhizobium* population. In one quarter of the samples no nodules were observed, and in another quarter the few nodules that were present were very small or senesc. The sampling of large, apparently effective, nodules resulted in the isolated and purification of fifty strains. These were presumed to be *R. phaseoli* based on colony and cellular morphology and a negative ketolactase test. Although there were several farms in which good nodulation was observed, it was concluded in these two important bean-growing areas of El Salvador, that overall, nitrogen fixation by the farmers' bean variety (in most cases Rojo de Seda), with the native *Rhizobium* population, was very poor.

**Strain screening**

The isolated strains, along with some checks from CIAT, were screened in the glasshouse in soils from bean-growing areas to identify those which were the most effective and competitive. 'Rojo de Seda' was inoculated with strains from two regions in soils from their respective regions. Total plant N and nodule weight are shown in Fig. 1. Both characters were poor in the soil from Candelaria de la Frontera. Nitrogen availability was probably not a factor limiting growth in this soil, as no response to mineral N was obtained, and therefore it was not possible to identify potentially good strains. However, effective strains were identified in the soil from Ilobasco, where nodulation and plant growth were improved with inoculation. The striking differences in nodulation as a result of inoculation were not correlated with the total plant N values ($r = 0.072$), probably because of differences in strain effectiveness. This indicates that nodule weight has limited value as a strain screening criterion in glasshouse conditions. Nodule number (data not shown) was no better.

The inoculation of 'RAB-204' in soil from Candelaria de la Frontera resulted in plant N increments that varied significantly according to the *Rhizobium* strain (Fig. 2). Although plant growth was poor, as in the previous trial, a significant response to fertilizer N was observed. The ranking was the same for the three strains common to the two trials in which Candelaria soils were used (Fig. 1, 2); that is, strain 632 was the best, 899 the poorest and 144 intermediate. The interaction of plant genotype by *Rhizobium* strain for total plant N was not significant ($P = 0.205$). This suggests that a strategy of testing a wider range of *Rhizobium* germplasm with a single bean genotype would be advantageous, at least in situation where similar varieties are grown throughout the region (in this case small-red-seede indeterminate beans predominate). Obviously a genotype with good fixation potential would permit better differentiation between strains. As in the case of 'Rojo de Seda' (Fig. 1), there was no significant correlation between nodulation parameters (nodule weight, nodule number) and plant N yield (Fig. 2). There was no difference between genotypes in nodule number (71 and 68 in 'Rojo de Seda' and 'RAB 204' respectively), however the nodules of 'Rojo de Seda' weighed significantly more ($P < 0.01$) than those of 'RAB-204' (75 mg and 62 mg respectively). The generally poor plant growth in soil from Candelaria de la Frontera was probably due to a deficiency of other nutrients accentuated by the small soil volume in the pots. This has been observed in strain screening experiments in soil cores (3), and for future trials it is recommended that macro and micro nutrients be applied even if they are not recommended in the field, to reach equivalent tissue levels.

**Field inoculation trials**

In 1986 farm trials were planned for both regions, Ilobasco and Candelaria de la Frontera; unfortunately it was not possible to plant the trial in Ilobasco. However, interesting strains (considering all evaluated factors) had already been chosen and inoculants prepared. The effect of inoculating the farmers' variety, Rojo de Seda, with six different *Rhizobium* strains (four from Ilobasco) was evaluated. Nodule number at 20 days after germination was significantly increased by inoculation (Table 1). By the 40 day harvest, 90 per cent of the nodules had senesced and differences between treatments were no longer apparent. This, and the observation in the farm surveys of predominantly senescent nodules at the mid-flowering stage, suggests that the duration of fixation in 'Rojo de Seda' is very short. As an estimate of vegetative vigor, shoot dry weight was determined at 40-days. Only the N-fertilized control was significantly more vigorous than the other treatments

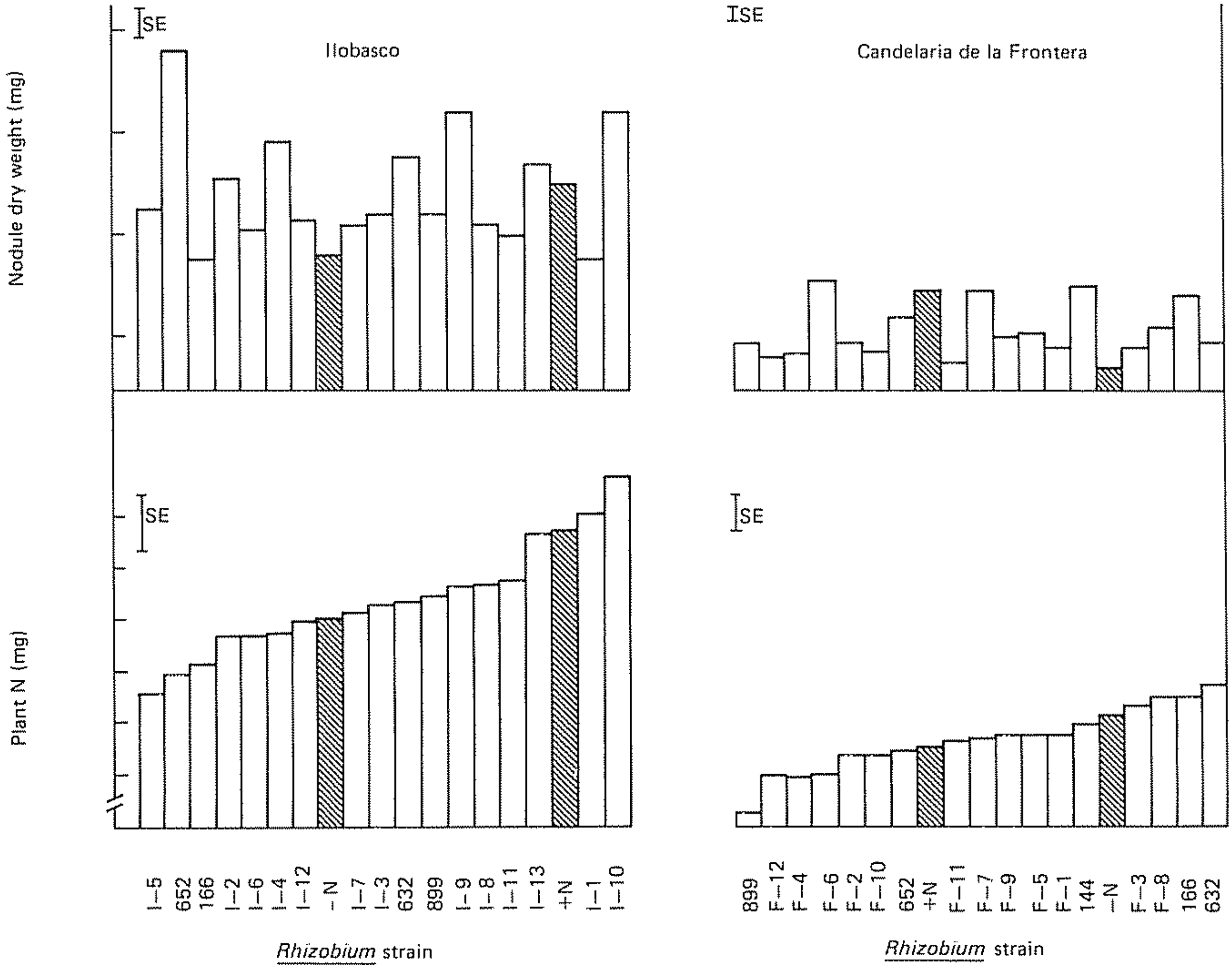


Fig 1 Nodule weight and plant nitrogen of the bean variety 'Rojo de Seda,' inoculated with 17 *Rhizobium* strains in each of two soils

Table 1). Treatment ranking for yield was similar to that for vigor but differences were not significant at the 5 per cent level. The pH of this soil was 5.2, levels of phosphorus were low, there was a drought towards the end of the season and part of one block was shaded by nearly trees; all of these factors contributed to the low yields and high experimental error in this trial. The results, however were considered sufficiently promising to warrant further trials the following season.

Four strains selected in the 1987 glasshouse screening trial and 'I-10', the best strain in the 1986 field trial, were used to inoculate the bean variety RAB-204 in a field trial. As in the previous experiment, nodulation at 20 days was increased as a result of inoculation (Table 2), but by the 50-day harvest the differences were no longer significant. The yield response of 'RAB-204' to nitrogen fertilizer was striking, confirming the importance of nitrogen as a production limiting factor in this area. The response to *Rhizobium* inoculation was also significant, depending on the inoculant strain used (Table 2). Inoculation with strain A-15 resulted in an increase in nodule number (as was observed in the glasshouse experiment (Fig. 2). However, grain yield was no better than with the native *Rhizobium* population. Further confirmation studies on the yield effect of inoculation with strains J-12 and P-22 are justified for this region of El Salvador.

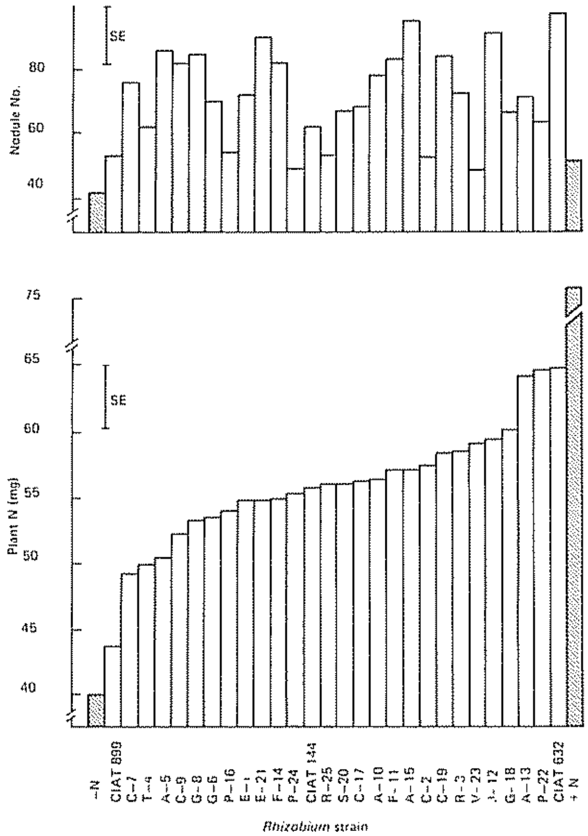


Fig 2. Nodule number and plant nitrogen of 'Rojo de Seda' and RAB-204 inoculated with 28 different *Rhizobium* strains in soil from Candelaria de la Frontera

Table 1. Nodulation, vigor and yield of 'Rojo de Seda' inoculated with different *Rhizobium* strains in Candelaria de la Frontera (1986) (Means of three repetitions).

| Treatment | Nodule No./plant-20 days | Shoot dry wt. g/plant-40 days | Yield kg/ha |
|---|---|---|---|
| +N | 3 c[1] | 7.59 a | 882 |
| I–10 | 43 a | 5.06 b | 732 |
| CIAT-632 | 45 a | 4.51 b | 695 |
| CIAT-899 | 46 a | 4.87 b | 563 |
| I–8 | 36 a | 4.59 b | 546 |
| I–2 | 48 a | 3.70 b | 465 |
| −N,−inoc | 19 b | 4.08 b | 406 |
| I–9 | 47 a | 4.36 b | 353 |

1 Duncan's multiple range test was used to separate means when the F-test was significant. Those means with different letters are significantly different ($P \leq 0.05$)

Table 2. Nodulation and yield 'RAB-204' inoculated with different *Rhizobium* strains in Candelaria de la Frontera (1987) (Means of three repetitions).

| Treatment | Nodule No./plant-20 days | Yield kg/ha |
|---|---|---|
| +N | 13 bc[1] | 2 302 a |
| J–12 | 19 abc | 1 915 a |
| P–22 | 16 abc | 1 619 ab |
| I–10 | 27 a | 1 496 ab |
| R–3 | 28 a | 1 449 ab |
| A–15 | 25 a | 982 b |
| −N | 12 c | 970 b |

1 Duncan's multiple range test was used to separate means when the F-test was significant. Those means with different letters are significantly different ($P \leq 0.05$).

## Bean variety evaluation

In order to optimize $N_2$ fixation by legume-rhizobium symbiosis, it is important to consider the nodulation and fixation potential of the legume genotype. Diversity in these characters has been observed between bean genotypes (9) and even between small-seeded, indeterminate bush beans of similar maturity (4-6). In 1987 an experiment was conducted to evaluate nodulation and responses to nitrogen fertilizer and *Rhizobium* inoculation of four new promising bean genotypes and the present farmers' commercial variety, Rojo de Seda. In the trial moisture was adequate, P fertilizer was applied and pesticides used as needed, resulting in high yields and significant N deficiency. All five genotypes responded to N fertilizer and the rankings under low and high mineral N availability were the same (Table 3). One would not expect this constant rank-

Table 3. Yield response of five bean varieties to nitrogen fertilizer and *Rhizobium* inoculation (1987).

| Bean | Yield (kg/ha) | | | |
|---|---|---|---|---|
| variety | Low N | Inoculated | High N | Means |
| RAB-310 | 1 762 | 2 914 | 3 104 | 2 593 a |
| Centa-Izalco | 2 046 | 2 044 | 3 158 | 2 416 a |
| RAB–404 | 1 754 | 2 661 | 2 667 | 2 360 ab |
| RAB–204 | 1 429 | 1 896 | 1 796 | 1 707 bc |
| Rojo de Seda | 1 276 a | 1 604 b | 1 658 | 1 513 c |
| means | 1 654 a | 2 224 b | 2 477 b | |

Varietal differences significant at $P < 0.05$; N treatment differences significant at $P < 0.01$, Interaction not significant at $P = 0.05$

ing if the genotypes were very different in their ability to fix nitrogen with the native *Rhizobium* population, unless yield potential and fixation potential were highly correlated No significant differences in nodulation under non-inoculated, low N conditions were observed at the 50-day harvest, and the differences in the inoculated treatment were minimal (Table 4). Because of the late evaluation, these data probably relate more to the character of late nodule senescence than to maximum nodulation potential of the bean genotypes. The inoculated (low N) treatment serves as an indicator of fixation potential of the genotypes without limitation of *Rhizobium* strains. Centa-Izalco was the only genotype that did not respond to inoculation, and yield in this treatment was less than non-N-limited potential (Table 3). With all other genotypes much of the N deficiency was overcome by improving the symbiosis through manipulation of the *Rhizobium* strain component. Further studies on a large number of farms in this region using farmers' levels of phosphorus and pesticides, are required to determine the economic potential of inoculation technology

Table 4. Nodulation at 50-days of five bean varieties inoculated with a mixture of the *Rhizobium* strains J–12, P–22 and R–3 (1987).

| Bean variety | Nodule No./plant |
|---|---|
| RAB–204 | 36 a |
| RAB–310 | 25 ab |
| Centa-Izalco | 16 ab |
| Rojo de Seda | 8 b |
| RAB–404 | 3 b |

Duncan's multiple range test, $P < 0.10$

Many trials have been undertaken to study the effect of *Rhizobium* inoculation on bean yields, with variable responses. In some cases nitrogen is not the yield-limiting factor, but another frequent cause of inoculation failure is the lack of competitiveness of the inoculant strains (7) Fundamental in this study was the evaluation of a large number of *Rhizobium* strains for their ability to induce a plant response in the presence of the native *Rhizobium* populations in local soils, that is, competitive and effective strains were selected. More strain screening is still needed for other bean-growing areas of El Salvador and it is important that potential for $N_2$ fixation be considered as one of the selection criteria as new bean germplasm is introduce.

## LITERATURE CITED


1. BLISS, F.A. 1984. Breeding for enhanced dinitrogen fixation potential of common bean. In Nitrogen fixation and $CO_2$ metabolism. Ed by P.W. Ludden; J.E Burris, New York, Elsevier Press. p. 303-310

2. CIAT (COL.). 1982. Annual report of the Bean Program 1981. Cali p. 153-157.

3. CIAT (COL.). 1985. Informe anual del Programa de Pastos Tropicales 1984 Cali. p. 156-159

4. CIAT (COL.). 1986. Annual report of the Bean Program 1985 Cali. p. 107-116

5. CIAT (COL.) 1987. Annual report of the Bean Program 1986. Cali. p. 153-162

6. CIAT (COL.) 1988. Annual report of the Bean Program 1987 Cali. p. 60-71

7. GRAHAM, P.H. 1981 Some problems of nodulation and symbiotic nitrogen fixation in *Phaseolus vulgaris* L.: A review. Field Crops Research 4:93-112.

8. GRAHAM, P.H.; HALLIDAY, J. 1977. Inoculation and nitrogen fixation in the genus *Phaseolus*. In Exploiting the legume-*Rhizobium* Symbiosis in Tropical Agriculture. Ed. by J.M Vincent *et al.* USA, University of Hawaii College of Tropical Agriculture Misc. Publication no. 145 p. 313-334.

9. GRAHAM, P.H.; TEMPLE, S.R. 1984 Selection for improved nitrogen fixation in *Glycine max* (L.) Merr. and *Phaseolus vulgaris* L. Plant and Soil 82:315-327.

10. GUAR, Y.D.; MARECKOVA, H. 1977. Absence of 3-ketoctose production by *Rhizobium phaseoli* Folia Microbiology 22:311-312

11. HUBBELL, D.H.; FREDERICK, L.R. 1976. Determinación de inoculación de *Rhizobium* y posibles problemas relacionados con el cultivo del frijol en El Salvador. Informe Universidad de Florida-CENTA. 4 p.

12. MINISTERIO DE AGRICULTURA Y GANADERIA, (EL SALVADOR). 1984 Anuario de estadística agropecuaria. San Salvador, Dirección de Economía Agropecuaria

13. VINCENT, J.M 1970 A manual for the practical study of root-nodule bacteria. Oxford, U.K. Blackwell Scientific Publ. 164 p.