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# Cultivar and Rhizobium strain effect on nitrogen fixation and transport in *Phaseolus vulgaris* L.

MARIANGELA HUNGRIA and MARIA C. P. NEVES
*EMBRAPA-Programa Nacional de Pesquisa em Biologia do Solo Km 47, Seropédica, 23851, Rio de Janeiro, Brazil*

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**Abstract**

The effects of Rhizobium strain and its interaction with plant cultivar were examined in glasshouse-grown *Phaseolus vulgaris* in two experiments where the physiological attributes defining the symbiotic efficiency were determined.

Strains of Rhizobium significantly affected nodulation, rates of N accumulation, partitioning of N within the mature shoot and remobilizaton of the N stored in the vegetative organs to the seeds.

The most efficient symbiosis (strain CO5 with Negro Argel), in comparison with the least efficient symbiosis (strain 127 K-17 with Venezuela-350) showed higher rates of $C_2H_2$ reduction from flowering to mid pod fill stage, evolved less hydrogen from nodules and showed higher rates of N transport as well as higher percentages of ureide-N in the xylem sap. At maturity, the best cultivar/strain association exceeded the total N accumulated in the seed and the harvest index of the poorest symbiosis in 88% and 20%, respectively. The other symbiotic combinations were intermediate in all characteristics.

Nitrogen accumulation in plant shoot showed highly significant correlation with acetylene reduction rates, nodule relative efficiency, total N transport in the xylem sap and percentage of N transported as ureides.

## Introduction

The process of biological fixation has a considerable requirement for energy and reducing power which is supplied by the respiratory catabolism of photosynthates produced by the host plant (Schubert and Ryle, 1980). As legume plants have the $C_3$ photosynthetic pathway, which is characterized by low rates of net photosynthesis, evidence exists that the supply of photosynthates often limits the process of nitrogen fixation (Hardy and Havelka, 1976). For this reason, the observation that there is a fall in nitrogenase activity during the grain-filling stage, the time when the nitrogen demand for grain production is greatest, has been attributed to the competition for available photosyntates between reproductive organs and the nodules (Atkins *et al.*, 1978; Lawn and Brun, 1974) and this may limit the yield potential of nodulated grain legumes. Other experiments, however, have shown that this decline in activity immediately after flowering does not necessarily occur (Hardy *et al.*, 1968; Mague and Burris, 1972) and that the process of nitrogen fixation can be sufficient to fulfill the demand for nitrogen during the reproductive growth phase (Israel, 1981; Nelson and Weaver, 1980).

In the symbiosis *Phaseolus vulgaris — Rhizobium leguminosarum* biovar *phaseoli*, both plant cultivars and Rhizobium strains can affect nodulation, nitrogenase activity and the accumulation of nitrogen in the vegetative and reproductive tissues (Franco and Döbereiner, 1967; Ruschel *et al.*, 1979), the rate of translocation of nitrogen in the xylem sap, and the partition of fixed nitrogen (Ruschel and Saito, 1977; Ruschel *et al.*, 1979; Ruschel *et al.*, 1982).

It follows that, in the selection of genotypes for

high seed production via maximization of biological nitrogen fixation in Phaseolus beans it is necessary to understand the factors connected with plant and/or Rhizobium strain which control the variation in nitrogen fixation during plant development, as well as the parameters related to the best symbiotic performance of cultivars and strains. With these objectives, two experiments were performed with five cultivars of Phaseolus beans and six strains of Rhizobium.

## Material and methods

### *Experiment 1*

The experiment was performed in the greenhouse at EMBRAPA-UAPNPBS, Km 47, Rio de Janeiro, and planted in May, 1982. Metal pots of 6 liter capacity were used, filled with a mixture of washed sand and vermiculite (1:2, v:v) and sterilized in an oven at 120°C for 36 h. The *Rhizobium leguminosarum* biovar *phaseoli* strains CO5 (CENA, Piracicaba, São Paulo, and 127 K-17 (Nitragin Co., USA) were grown in YMA medium (Vincent, 1970) for 4 days at 28°C with agitation. Seeds of *Phaseolus vulgaris* L. cultivars Negro Argel and Venezuela 350 were surface sterilized with 0.2% $H_gC1_2$ (Vincent, 1970), treated with 1 ml of inoculant (approximately $10^8$ cells · $ml^{-1}$) for each 15 seeds and incubated for one hour. Five seeds were planted per pot and covered with a layer of approximately 3 cm of sterile sand. Seven days after emergency (DAE) plants were thinned to 2 plants per pot.

Plants were irrigated daily based on the weight of 5 pots taken from each treatment. Every 10 days, 250 ml of N free nutrient solution (modified from McKnight, 1949) of the following composition were added: $CaSO_4 \cdot 2H_2O$ (58.1 m*M*); $MgSO_4 \cdot 7H_2O$ (8.1 m*M*); $KH_2PO_4$ (14.7 m*M*); KCl (40.2 m*M*); $H_3BO_3$ (462 $\mu M$); $MnSO_4 \cdot 4H_2O$ (9.1 $\mu M$); $ZnSO_4 \cdot 7H_2O$ (7.6 $\mu M$); $CuSO_4 \cdot 5H_2O$ (3.2 $\mu M$); $H_2MoO_4 \cdot 4H_2O$ (5.0 $\mu M$); and $FeCl_3$ (82.9 $\mu M$); pH 6.0 to 6.2.

The experiment was layed out in a complete randomized block design with 8 replicates, 4 treatments and 11 harvests, totaling 352 pots. Harvests were performed at weekly intervals starting at 7 DAE.

At each harvest, 4 pots (each with two plants) were used to determine the nitrogenase activity and 4 pots were used to determine hydrogen evolution. Each sap sample was collected from two pots, (4 plants). Subsequently, nodules were removed from the roots and the plants separated into leaves, stems, pods, seeds, roots and nodules, and dried to constant weight in an oven at 60° to 70°C, and the tissues were analysed for total N.

### *Experiment 2*

To expand the data obtained in Experiment I, a second experiment was planted in October 1983, in the greenhouse, using Leonard jars (Vincent, 1970). Five bean cultivars (Negro Argel, Carioca, Venezuela 350, Costa Rica and Rio Tibagi), were inoculated separately with six strains of *Rhizobium leguminosarum* biovar *phaseoli* (CO5 —CENA, São Paulo; $FP_2$—EMBRAPA—UAPNPBS, Rio de Janeiro; SEMIA 487—IPAGRO, Porto Alegre; 127 K-17—Nitragin Co., USA; CIAT 255 and CIAT 727—CIAT, Cali-Colombia). The experimental design was a factorial of 5 cultivars and 6 strains with 4 blocks and 2 harvests, one at flowering (35 to 38 DAE), and the other one at mid-pod-fill (50 DAE). The experiment was performed as described in experiment 1. Every week the plants received nutrient solution devoid of mineral N and containing: KCl (2.00 m*M*); $K_2HPO_4$ (2.20 m*M*); $CaSO_4 \cdot 2H_2O$ (2.00 m*M*); $KH_2PO_4$ (0.29 m*M*);$MgSO_4 \cdot 7H_2O$ (2.0 m*M*); $ZnSO_4 \cdot 7H_2O$ (0.76 $\mu M$); $MnSO_4 \cdot 2H_2O$ (0.09 $\mu M$); $(NH_4)_6Mo_7O_2 \cdot 4H_2O$ (0.008 $\mu M$); $H_3BO_3$ (11.56 $\mu M$); $FeSO_4$ (18 $\mu M$), pH 6.0–6.2. At flowering the sap was collected from four pots (each one with two plants) for 15 minutes. After this, one plant from each pot was used to determine nitrogenase activity and the other one to determine the hydrogen evolution by the nodules. The plants were separated in shoots, roots and nodules, dried to constant weight and the shoots were analysed for total N. At 50 DAE the stems, leaves and pods were analysed for total N.

### *Analysis*

For the determination of nitrogenase activity in Experiment 1 the acetylene reduction activity of

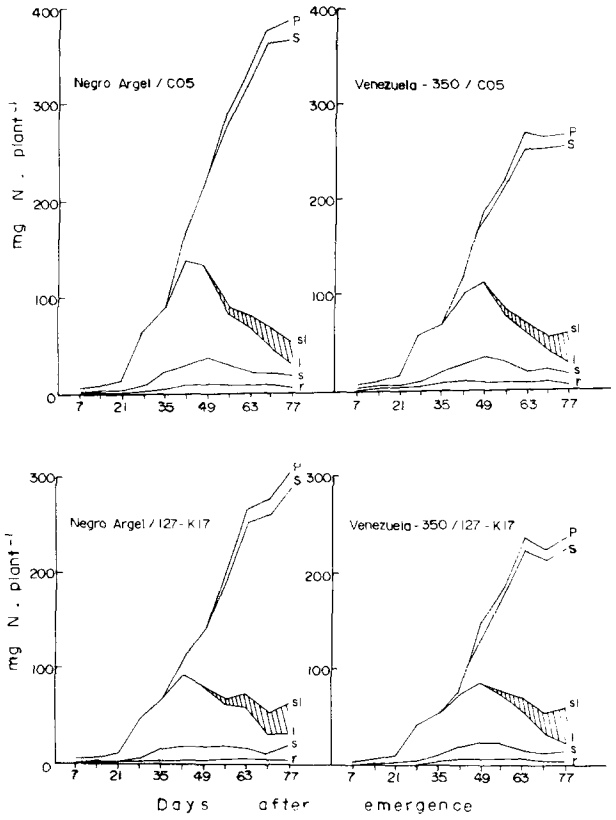


*Fig. 1.* Effect of plant cultivar and Rhizobium strain on nitrogen contents of vegetative and reproductive components during growth of Phaseolus beans. Means of 8 replicates; **P** (pod walls), **S** (seeds), **SL** (Senesced leaves), **L** (leaves), **S** (stems), **r** (roots); (Data from Experiment 1).

excised roots was used (Mague and Burris, 1972). Roots, separated from shoots at the first cotiledonary node, were sealed into a 250 ml bottle and incubated for 30 minutes in an atmosphere containing 12% acetylene. To determine whether the nitrogenase activity was affected by the presence of acetylene (Minchin *et al.*, 1983) an additional experiment was performed with the same plant cultivars and Rhizobium strains. A continuous flow system was used consisting of a glass chamber flushed with an air stream containing 12% acetylene at a rate of 100 ml · min$^{-1}$. Samples were taken after 1, 3, 4, 5, 7, 10, 15 and 30 minutes of incubation. Decreases in nitrogenase activity of only 4.2, 4.7, 4.3 and 0% respectively were observed in the systems Negro Argel/CO5, Negro Argel/127 K-17, Venezuela 350/CO5 and Venezuela 350/127 K-17, showing that the standard acetylene reduction assay (Mague and Burris, 1972), could be used

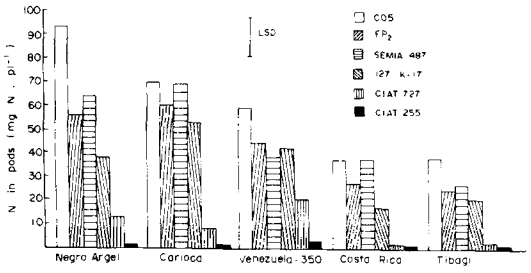


*Fig. 2.* Effects of interaction between Rhizobium strain and bean cultivar on N accumulation in pods at 50 days after emergence. Means of 4 replicates; *vertical bars* denote LSD between treatment means at $P = 0.01$ (Data from Experiment 2).

without incurring substantial error. In the second experiment the continuous flow technique was used in all determinations. Ethylene was analysed in a chromatograph as described before (Hungria and Neves, 1987).

Hydrogen evolution was determined on nodules detached from the roots with small segments of root (0,5 cm), and incubated for 30 minutes. Samples were analysed as described before (Hungria and Neves, 1987).

The relative efficiency of the electrons used by the nitrogenase was determined according to the equation:

$$RE = 1 - \frac{H_2 \text{ evolved (air)}}{C_2H_2 \text{ reduced}}$$ (Schubert and Evans, 1976)

Xylem sap was collected from plants decapitated at the cotiledonary node. The root stumps were rinsed and dried with tissue paper. The exuded sap was collected in calibrated microcapillaries (20 and 50 $\mu$l) for 15 minutes so that the exudation rate could be calculated. The sap was maintained at $-20$°C until analysis. Determinations of ureide-N, amino-N, ammonium-N, amide-N and total-N in the xylem sap were made on aliquots of 5 $\mu$l of sap, according to Boddey *et al.* (1987).

## Results

Throughout plant growth in experiment 1, the symbiotic combination of cultivar Negro Argel with the Rhizobium strain CO5 out-yielded the other combinations in terms of total N (Fig. 1) in both the vegetative and reproductive parts of the plant. There were significant effects of plant cul-

*Table 1.* Interaction between bean cultivars and Rhizobium strains on plant growth, seed yield, N partitioning and N lost in senesced leaves at 77 days after emergence. Values are means of 8 replicates, values followed by the same letter are not different at $P = 0.01$ (Tukey's test)

| Parameter | Negro Argel | | Venezuela 350 | |
|---|---|---|---|---|
| | C-05 | 127 K-17 | C-05 | 127 K-17 |
| Plant dry weight ($g \cdot pl^{-1}$) | 18.68a | 15.66a, b | 14.60b | 13.50b |
| Total N in plant ($mg\,N \cdot pl^{-1}$) | 375.15a | 307.85b | 262.04b, c | 238.95c |
| Seed dry weight ($g \cdot pl^{-1}$) | 9.47a | 7.36b | 7.22b | 5.91c |
| % N in the seeds | 3.28a | 3.07a, b | 2.73c | 2.80b, c |
| Total N in seeds ($mg\,N \cdot pl^{-1}$) | 310.59a | 225.72b | 197.72b | 165.45c |
| Harvest Index (Seed dry weight: Plant dry weight) | 0.51a | 0.47b | 0.49b | 0.44c |
| N Harvest index (N in seeds: N in plant) | 0.83a | 0.73b | 0.75b | 0.69c |
| Senesced leaves dry weight ($g \cdot pl^{-1}$) | 4.41a | 3.81a, b | 3.69a, b | 3.37b |
| N concentration in senesced leaves (%) | 0.68c | 1.14b | 1.08b | 1.62a |
| % N lost in senesced leaves (N in senesced leaves: N in plant) | 7.80c | 13.60b | 15.72b | 22.86a |

tivar and Rhizobium strain on dry matter and total N in leaves, stems and pods. This was also observed in experiment 2, where the best cultivar/ strain combination (Negro Argel/CO5) exceeded 45 times the pod N of the poorest symbiotic combination (Rio Tibagi/CIAT 255) (Fig. 2).

In Experiment 1, there were no significant differences in dry weight of senesced leaves between the symbiotic systems at the final harvest (Table 1). However, there were significant effects of cultivar, strain and also strain/cultivar interaction on the concentration of N in the senesced leaves. It follows therefore that the remobilization of nitrogen from the leaves was influenced by both plant cultivar and Rhizobium strain and the percentage of the total N lost in senesced leaves ( N in senesced leaves/total N in plant) in the association Venezuela 350/127 K-17 was 140% greater than in the Negro Argel/ CO5 association (Table 1).

Both plant cultivar and Rhizobium strain affec-

*Table 2.* Rhizobium strains and bean cultivars effects on nodulation, nodule efficiency and total N in shoots (leaves + stems + pods) at 35 days after emergence and on total N in pods at 50 days

| Treatments | 35 DAE (flowering) | | | 50 DAE |
|---|---|---|---|---|
| | Nodule dry weight | N in shoots | Nodule efficiency | N in pods |
| | ($g \cdot pl^{-1}$) | ($mg\,N \cdot pl^{-1}$) | ($mg\,N \cdot g\,nod^{-1}$) | ($mg\,N \cdot pl^{-1}$) |
| *Rhizobium strains* | | | | |
| C-05 | 0.479b[a] | 183.37a | 382.82a | 58.04a |
| $FP_2$ | 0.734a | 102.72b | 139.96b | 42.58b |
| SEMIA 487 | 0.636ab | 95.74b | 150.53b | 45.96b |
| 127 K-17 | 0.634ab | 77.60c | 122.40b | 33.78c |
| CIAT 727 | 0.288c | 21.97d | 76.28c | 8.60d |
| CIAT 255 | 0.133c | 6.06e | 45.56c | 0.96e |
| *Bean cultivars* | | | | |
| Negro Argel | 0.541b[b] | 116.85a | 215.99a | 44.08a |
| Carioca | 0.377c | 92.04b | 244.14a | 45.52a |
| Venezuela 350 | 0.636a | 80.47c | 126.52b | 33.85b |
| Costa Rica | 0.442bc | 55.43d | 125.41b | 18.50c |
| Rio Tibagi | 0.424c | 61.43d | 144.88b | 18.31c |

[a] Values followed by the same letter are not significantly different at $P = 0.01$, averages of 20 repetitions (Tukey's test). Rhizobium strains were inoculated in five bean cultivars.

[b] Values followed by the same letter are not significantly different at $P = 0.01$, averages of 24 repetitions (Tukey's test). Bean cultivars were inoculated with five Rhizobium strains.

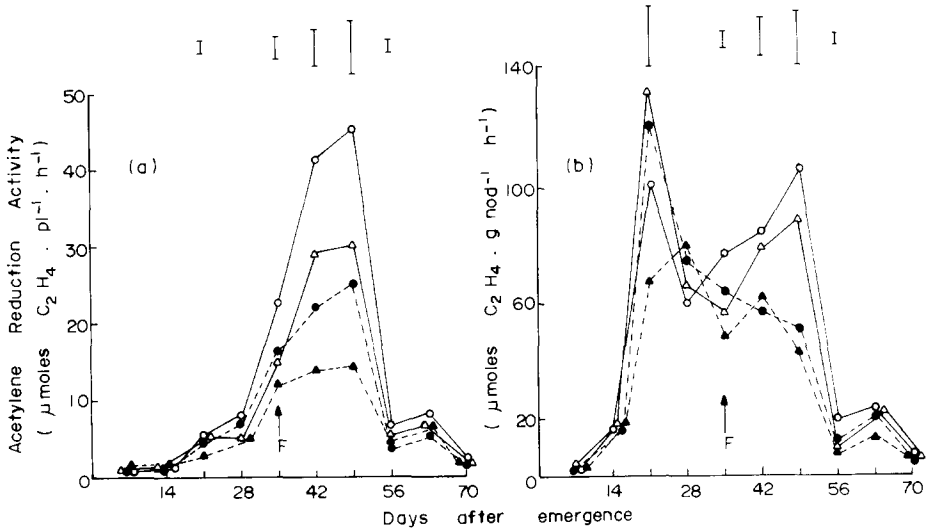


*Fig. 3.* Changes in the (a) total and (b) specific acetylene reduction during growth of *Phaseolus vulgaris* cultivars Negro Argel (○●) and Venezuela-(△▲) inoculated with strain CO5 (○△) or 127 K−17 (●▲). Values are means of 4 replicates; *vertical bars* denote LSD between treatment means at $P = 0.01$; ↑ denotes time of first flowering (Data from Experiment 1).

ted the final harvest index and the nitrogen harvest index (Table 1). The symbiosis Negro Argel/CO5 accumulated 78% more seed N than Venezuela 350/127 K-17, which was the result of a 60% increase in seed dry weight and a 17% increase in the N concentration in the grain.

During plant growth, nodule mass peaked at 42 DAE (data not shown), immediately after flowering, with a small secondary nodule production at the grain filling stage (63 DAE), but these nodules were smaller and localized at the root tips. In Experiment 2, although plants inoculated with strain CO5 had a relatively small nodule mass, they accumulated more N in shoot tissues (Table 2), and the nodule efficiency (mg N fixed · g nodule$^{-1}$) was 2.7 and 2.5 times greater than the plants inoculated with the strains $FP_2$ and SEMIA 487, respectively. The various plant cultivars also accumulated different quantities of N in the tissues, and Negro Argel and Carioca had higher nodule efficiency (Table 2) than other cultivars.

Total nitrogenase activity increased immediately after flowering (35 DAE) and reached maximum activity during mid pod filling stage, 49 DAE (Fig. 3). The effect of both strains and cultivars on total and specific nitrogenase activity was confirmed in Experiment 2 (Table 3). Hydrogen evolution by the nodules (Fig. 4) closely followed the pattern of nitrogenase activity, reaching maximum evolution at 49 DAE. Hydrogen evolution was greatest for those symbioses which accumulated least N, particularly during the phase of highest nitrogenase activity (between 35 and 56 DAE). Effects of both strains and cultivars on hydrogen evolution were confirmed in Experiment 2 (Table 3).

There was a decrease in the relative efficiency (RE) immediately after flowering (Fig. 5) and both strains and cultivars affected the RE. In Experiment 2, RE ranged from 0.37 (Rio Tibagi/CIAT 255) to 0.96 (Negro Argel and Carioca/CO5), showing that some symbiotic systems can waste much of their electron flux and ATP via this process (Table 3).

As to the transport of N in the xylem sap (Experiment 1), when N concentration alone was considered, it was not always possible to detect differences between cultivars and strains (Data not shown). However, when the rate of N exudation (N concentration × xylem exudation rate) was considered, differences were observed between the symbiotic systems, especially during the period of highest nitrogen fixation rates (Fig. 6). Most of the N was transported in the form of ureides, allantoin and allantoic acid (Fig. 7), with the allantoic acid constituting from 52 to 88% of the total ureides (data not shown). There was also a significant effect of the plant cultivar and the Rhizobium strain on the sap composition. The most efficient combina-

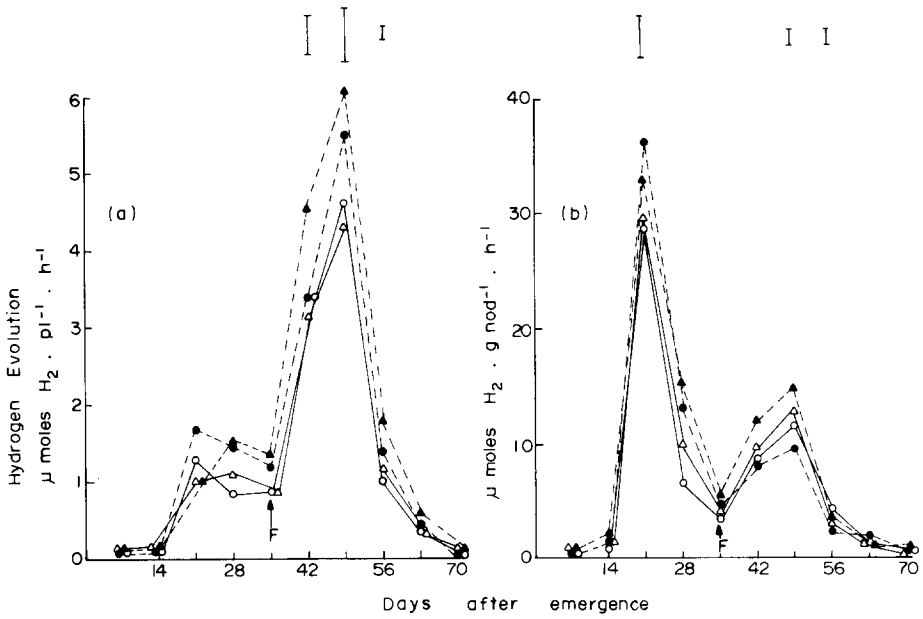


*Fig. 4.* Changes in (**a**) total and (**b**) specific hydrogen evolution from nodules during growth of *Phaseolus vulgaris* cultivar Negro Argel (○●) and Venezuela-350 (△▲) inoculated with strain CO5 (○△) or 127 K-17 (●▲). Values are means of 4 replicates; *vertical bars* denote LSD between treatment means at $P = 0.01$; ↑ denotes time of first flowering. (Data from Experiment 1).

*Table 3.* Rhizobium strains and bean cultivars effects on nitrogenase activity, hydrogen evolution and relative efficiency (RE) of nitrogenase at 35 days after emergence

| Treatments | Total activity ($\mu$moles· $pl^{-1}$· $h^{-1}$) | | Specific activity ($\mu$moles·$pl^{-1}$· $h^{-1}$) | | RE |
|---|---|---|---|---|---|
| | $C_2H_4$ | $H_2$ | $C_2H_4$ | $H_2$ | |
| *Rhizobium strains* | | | | | |
| C-05 | 10.96ab[a] | 1.47c | 25.48a | 3.11b | 0.88a |
| $FP_2$ | 13.24a | 2.33b | 19.76b | 3.44b | 0.83a |
| SEMIA 487 | 11.28ab | 2.28b | 19.28b | 3.98b | 0.79a |
| 127 K-17 | 9.82b | 3.29a | 14.92c | 5.71a | 0.62b |
| CIAT 727 | 2.49c | 1.52c | 9.02d | 5.07a | 0.44c |
| CIAT 255 | 0.64c | 0.39d | 6.66e | 3.80b | 0.43c |
| *Bean cultivars* | | | | | |
| Negro Argel | 10.14a[b] | 1.25d | 16.32b | 2.52c | 0.85a |
| Carioca | 7.83b | 1.56c | 18.55a | 4.78b | 0.74b |
| Venezuela 350 | 9.10ab | 2.42a | 15.34b | 4.02b | 0.74b |
| Costa Rica | 8.76ab | 2.21ab | 19.17b | 6.14b | 0.68b |
| Rio Tibagi | 4.50c | 1.96b | 9.78c | 4.74b | 0.52c |

[a] Values followed by the same letter are not significantly different at $P = 0.01$, averages of 20 repetitions (Tukey's test). Rhizobium strains were inoculated in five bean cultivars.

[b] Values followed by the same letter are not significantly different at $P = 0.01$, averages of 24 repetitions (Tukey's test). Bean cultivars were inoculated with five Rhizobium strains.

tion (Negro Argel with strain CO5) showed higher ureide concentration and lower rates of amino acid transport. No differences were found in the amide-N and ammonium-N concentrations (Fig. 7). The

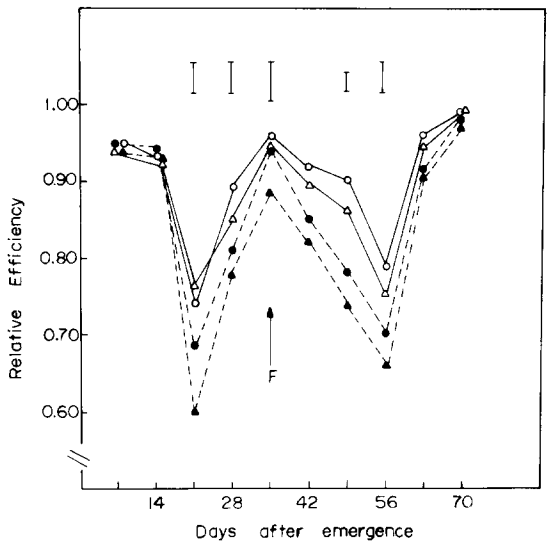


*Fig. 5.* Changes in the relative efficiency of nodules during growth of *Phaseolus vulgaris* cultivar Negro Argel (○●) and Venezuela-350 (△▲) inoculated with strain CO5 (○△) or 127 K-17 (●▲). Values are means of 4 replicates; *vertical bars* denote LSD between treatment means at $P = 0.01$; ↑ denotes time of first flowering. (Data from Experiment 1).

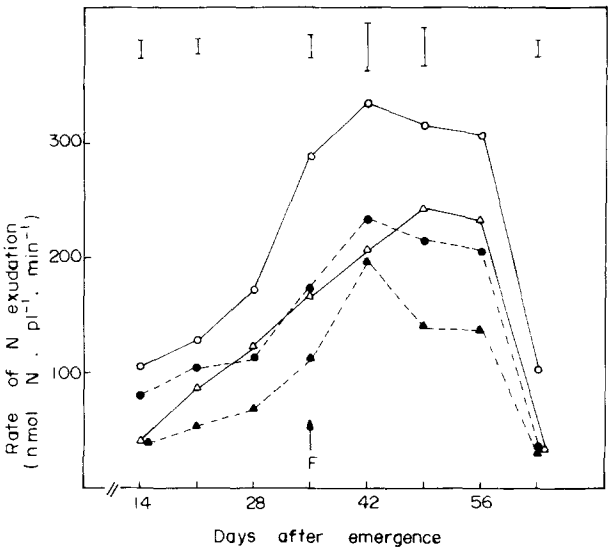


*Fig. 6.* Changes in the rate of N transport in xylem bleeding sap during growth of *Phaseolus vulgaris* cultivar Negro Argel (○●) and Venezuela-350 (△▲) inoculated with strain CO5 (○△) or 127 K-17 (●▲). Values are means of 4 replicates; *vertical bars* denote LSD between treatment means at $P = 0.01$; ↑ denotes time of first flowering. (Data from Experiment 1).

use of more symbiotic combinations in Experiment 2 confirmed the role of both plant cultivars and rhizobium strains not only in the rate of N exudation in the xylem sap but also in the xylem sap composition (Table 4). Plants inoculated with strain CO5 transported 77% more ureide-N than those inoculated with CIAT 255, and the cultivar Negro Argel transported 16% more ureide-N than Rio Tibagi.

A comparison was made between the correlations of various parameters with the total N accumulated by the plants at all harvests in Experiment 1 (Table 5). Despite the problems associated with the acetylene reduction technique (Minchin *et al.*, 1983), a good correlation was found between the acetylene reduction acitivity and N accumulated in plant tissues, but only after 35 DAE, and the correlation with the RE was greater (Table 5). Most surprising was the highly significant correlation of RE at all harvest between 21 and 63 DAE with the total N in seeds and especially with the N harvest index at the final harvest. For example, the correlation between RE at 35 DAE and the seed N and N harvest index at maturity (77 DAE) was respectively of 0.818** and 0.850**. In Experiment 2, there was also a correlation between total nitrogenase activity ($r = 0.707$**) or specific activity ($r = 0.745$**) and N accumulated in shoot at flowering, but the correlation between RE ($r = 0.823$**) and N accumulated in shoot was higher and statistically different.

The analysis of N compounds in the xylem sap has been suggested to be an efficient and practical technique to select the best symbiotic systems (Thomas *et al.*, 1984). It should be noted in the results of Experiment 1 that the N concentration in the

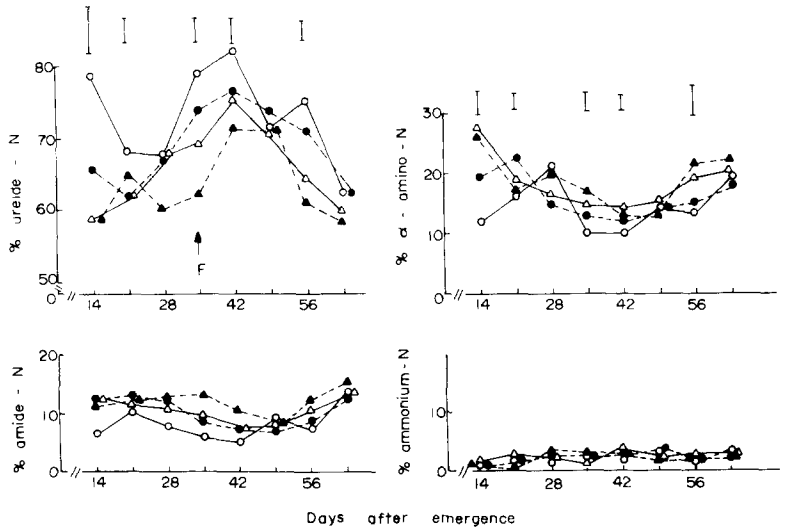


*Fig. 7.* Changes in the N composition of the xylem bleeding sap during growth of *Phaseolus vulgaris* cultivar Negro Argel (○●) and Venezuela-350 (△▲) inoculated with strain CO5 (○△) or 127 K-17 (●▲). Values are means of 4 replicates; *vertical bars* denote LSD between treatment means at $P = 0.01$; ↑ denotes time of first flowering. (Data from Experiment 1).

*Table 4.* Rhizobium strains and bean cultivars effects on N transport in the xylem sap at 35 days after emergence

| Treatments | N concentration | Xylem sap exudation | Rate of N transport | Ureide-N |
|---|---|---|---|---|
| | ($\mu$moles N · ml$^{-1}$) | ($\mu$l · pl$^{-1}$ · min$^{-1}$) | (nmol N · pl$^{-1}$ · min$^{-1}$) | % |
| *Rhizobium strains* | | | | |
| C-05 | 41.83a[a] | 1.13a | 47.31a | 90.55a |
| $FP_2$ | 39.49a | 0.89b | 35.13b | 85.26a |
| SEMIA 487 | 32.06b | 1.02a | 32.74b | 87.11a |
| 127 K-17 | 29.38b | 0.79c | 23.20c | 78.30b |
| CIAT 727 | 29.57b | 0.43d | 12.84d | 56.16c |
| CIAT 255 | 16.82c | 0.31e | 5.20e | 51.10c |
| *Bean cultivars* | | | | |
| Negro Argel | 34.65a[b] | 1.19a | 41.31a | 80.16a |
| Carioca | 39.25a | 1.03a | 40.51a | 79.51a |
| Venezuela 350 | 27.43b | 0.92a | 25.17b | 73.79b |
| Costa Rica | 24.96b | 0.61b | 15.25c | 71.42b |
| Rio Tibagi | 26.33b | 0.59b | 15.51c | 68.85c |

[a] Values followed by the same letter are not significantly different at $P = 0.01$, averages of 20 repetitions (Tukey's test). Rhizobium strains were inoculated in five bean cultivars.

[b] Values followed by the same letter are not significantly different at $P = 0.01$, averages of 24 repetitions (Tukey's test). Bean cultivars were inoculated with five Rhizobium strains.

xylem sap was not always a good parameter, but the best correlations with total N in the plant were obtained with the rate of N exudation (from 28 DAE onwards) and with the % of N as ureides at 35, 42 and 56 DAE (Table 5). In the same way, in Experiment 2, there was a significant correlation between the rate of N transported in the xylem sap at flowering (35 DAE) and the N accumulated in shoots at this time (r = 0.882**), as well as with the N content of pods at 50 DAE (r = 0.949**).

In Experiment 1, high correlations were observed between the % of N as ureides in the xylem sap (at any given harvest from 28 to 63 DAE, except for 49 DAE) and the N accumulated in tissues (Table 5) as well as with the N in the pods or the N harvest index. Fig. 8 displays these correlations with the concentration of ureides at flowering, indicating an effect of ureides in the N partitioning within the shoot favouring seed production. These results were confirmed in Experiment 2, where there was a correlation between the % ureide-N in the xylem sap at flowering and the total N in shoots of the various symbiotic systems (r = 0.892**) or the N in pods at 50 DAE (r = 0.907**), indicating that

*Table 5.* Relationship between (X) acetylene reduction, relative efficiency (RE) and N in the xylem sap and (Y) N increments in plant tissues during plant growth cycle

| Parameter (X) | (Y) N increments in plant | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Days after emergence | | | | | | | |
| | 14 | 21 | 28 | 35 | 42 | 49 | 56 | 63 |
| Acetylene reduction ($\mu$moles $C_2H_4$ · pl$^{-1}$ · h$^{-1}$) | n.s. | n.s. | n.s. | 0.842**[a] | 0.809** | 0.901** | 0.600** | 0.902** |
| Relative efficiency | n.s. | 0.845** | 0.894** | 0.901** | 0.841** | 0.909** | 0.900** | 0.915** |
| N concentratin in xylem sap ($\mu$mol N · ml$^{-1}$) | 0.635** | n.s. | n.s. | 0.888** | 0.540** | 0.700** | 0.760** | 0.940** |
| Rate of N exudation in the xylem sap (nmol N · pl$^{-1}$ · min$^{-1}$) | 0.606** | n.s. | 0.946** | 0.926** | 0.952** | 0.928** | 0.930** | 0.950** |
| % Ureide-N in the xylem sap | 0.510** | n.s. | 0.700** | 0.926** | 0.946** | n.s. | 0.935** | 0.737** |

[a] *and **indicate statistical differences at P = 0.05 and P = 0.01; n.s.—not significative

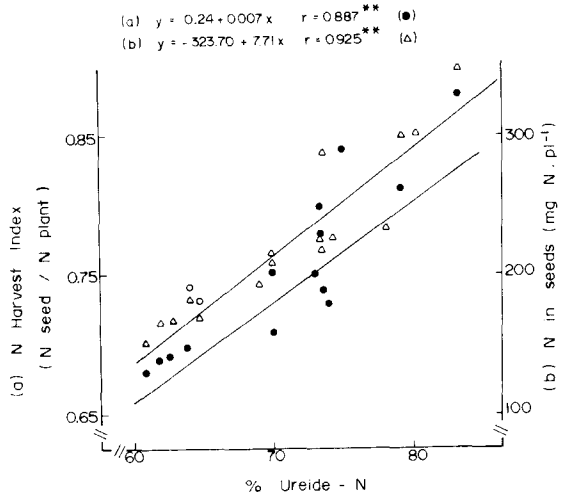


*Fig. 8.* Correlation between the ureide-N in the xylem sap at 35 days after emergence (DAE) and (a) N harvest index and (b) seed N at 77 DAE (Data from Experiment 1).

the % N as ureides in the sap can be a good parameter to evaluate symbiotic performance.

## Discussion

The observed interaction between plant cultivars and Rhizobium strains in the dry weight, total N of the vegetative and reproductive plant tissues, partition of N to the seeds, as well as in the nodule efficiency, confirms the results obtained before (Franco and Döbereiner, 1967; Hungria and Neves, 1987). It demonstrated the importance of considering both the host plant cultivar and the Rhizobium strain in the selection programs for higher $N_2$ fixation in Phaseolus beans as well as the need to take into account seed N and not only the total N accumulated in the plants.

The effect of host plant cultivar on the harvest index or on the N harvest index has already been reported in Phaseolus (Hungria and Neves, 1987; Ruschel and Saito, 1977; Ruschel *et al.*, 1979) and soybean (Israel, 1981) and the effect of the Rhizobium strain has been observed in beans (Hungria and Neves, 1987) and soybeans (Neves *et al.*, 1985), indicating differences in N metabolism.

The differences obtained in N harvest index between cultivars and strains can be attributed both to the direct transport of N to the seeds as well as to differences in the remobilization of N from the vegetative plant parts to the seeds. The latter possibility is illustrated by the large differences found between the symbiotic systems in the N lost in senesced leaves.

An increase in nitrogenase activity was observed after flowering showing that, at least in greenhouse conditions, nitrogen fixation can provide N directly for the seed nutrition. Some authors have observed a decline in nitrogenase activity immediatly after flowering, which was attributed to a decrease in the availability of C to the nodule metabolism, due to the competition for C by the developing pods (Franco *et al.*, 1979; Lawn and Brun, 1974). However, in this experiment, the pods represented, at 42 DAE, only 21% of the total plant dry weight and, therefore, could not have competed greatly for C during this period. Furthermore, pods can even stimulate nitrogen fixation (Hungria and Neves, 1986; Peat *et al.*, 1981) possibly due to a sink effect. Furthermore, Streeter (1981) showed that the decline in nitrogenase activity occured even when it was still possible to detect considerable quantities of C in the nodules. After 56 DAE, when pods represented 51% of plant dry weight, there was an abrupt fall in nodule activity, that could either be attributed to a competition for available photosynthates at this stage of growth, or to a hormonal effect (Malik, 1983).

The highest rates of hydrogen evolution by the nodules were not related to the highest rates of nitrogenase activity, probably indicating the activity of an uptake hydrogenase in the most efficient symbioses. Initially, it was believed that the hydrogen metabolism was controlled only by the Rhizobium strain and that only the bacteria contained the genetic information for the synthesis of hydrogenase (Carter *et al.*, 1978; Schubert *et al.*, 1978). The observations from this study and also from other recent ones (Bedmar *et al.*, 1983; Miller and Sirois, 1982) indicate, however, that the host plant can also influence the hydrogen metabolism.

The values of RE among the various cultivar and strain combinations were between 0.37 and 0.96 (Experiment 2), showing that certain bean/Rhizobium combinations may waste much of their electron flux through hydrogen evolution, as it was also shown by Saito *et al.* (1980) and Pacovsky *et al.* (1984). Therefore, the RE found in beans can be much lower than the RE of 0.99 found with efficient strains in cowpea (Schubert and Evans, 1976) and soybeans (Carter *et al.*, 1978).

The best performance of both bean cultivars and Rhizobium strains in terms of nitrogen fixation was correlated with the greatest rates of N exudation in the xylem sap, as well as with the highest proportion of ureides in the xylem sap. Thus, the strain effects on ureide production confirm the results previously obtained in soybeans (Neves *et al.*, 1985) and indicate that the Rhizobium strain can also affect the assimilation in nodule cytosol and the subsequent transport of N, but in contrast to the observations that in soybeans the most efficient strains transport more aspargine and glutamine (Israel and McClure, 1980; Minasawa *et al.*, 1983).

The observed increase in the transport of ureides during the period of greatest nitrogenase activity, has been previously reported (Cookson *et al.*, 1980). This may be linked to an economy of C, as in this period it could be necessary to incorporate more ammonia without further increasing the demand for C skeletons and the C/N ratio equal to 1 in ureides (Atkins *et al.*, 1978; Thomas and Schrader, 1981a) represents the maximum efficiency of C usage for transport.

Recently, it was observed (Rainbird *et al.*, 1984) that although the ureides represent the greatest proportion of the N transported in the xylem of soybeans, they do not constitute the main source of N to the embryo. Probably the allantoinase and allantoicase activity in stems and developing fruits (Herridge *et al.*, 1978; Thomas and Schrader, 1981b) permits the decomposition and reassimilation of the nitrogen derived form ureides into other N compounds which are used for the nutrition of the seeds.

The results obtained in these two experiments confirm the suggestions (Hungria and Neves, 1987; Neves *et al.*, 1985) that the ureide-N can be more easily incorporated in seed protein, as a result of either a higher remobilization of stored ureide-N in the vegetative parts or to a more direct or faster incorporation into the seeds.

The results observed in this experiment showed that both plant cultivar and Rhizobium strains can affect a great range of physiological parameters, that begin in the first steps of $N_2$ fixation and assimilation in the nodules until the incorporation of N in seeds. The elucidation of the complexity of the relationship of Rhizobium strains with plant physiology observed in this paper will lead to the comprehension of the apparently complicated Rhizobium bean symbiosis and result in symbiotic combinations which will become independent of nitrogen fertilizer.

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