

Acetylene reduction, hydrogen evolution and nodule respiration in *Phaseolus vulgaris*

M. Hungria¹ and A.P. Ruschel²

¹ EMBRAPA-UAPNPBS, Km 47, Antiga Rio-S. Paulo, 23851, Seropédica, Rio de Janeiro, Brazil

² EMBRAPA-CNPAP, Cx. Postal 179, 70000, Goiânia, Goiás, Brazil

Summary. In an experiment performed under greenhouse conditions, four cultivars of *Phaseolus vulgaris* L. (Venezuela-350; Aroana; Moruna; Carioca) were inoculated with three *Rhizobium leguminosarum* biovar *phaseoli* strains (C-05; C-40 = CIAT 255; C-89 = CIAT 55) and were fertilized with an N-free mineral nutrient solution. The plants were harvested 25, 40, and 55 days after emergence and the following parameters were evaluated: Nitrogenase activity of nodulated roots, H₂ evolution by the nodules; relative efficiency of nitrogenase; respiration rates of nodulated roots and detached nodules; dry weight and total N of stems, leaves, pods, roots, and nodules. Generally the bean cultivar, *Rhizobium* strain, had an effect and there was an interaction effect with both symbiotic partners, on all parameters. On average, nodules represented 23% of total root respiration but the best symbiotic combinations showed lower ratios of C respired to N fixed. The maximum N-assimilation rate (between 40 and 55 days after emergence) of 11.93 mg N plant⁻¹ day⁻¹ occurred with the symbiotic combination of Carioca × C-05, while the poorest rate of 0.55 mg N plant⁻¹ day⁻¹ was recorded with Venezuela-350 × C-89. The best symbiotic combinations always showed the highest relative nitrogenase efficiency, but the differences in N₂-fixation rates cannot be explained solely in terms of conservation of energy by recycling of H₂. This requires further investigation.

Key words: *Phaseolus vulgaris* – *Rhizobium* – Nitrogenase – Hydrogenase – Nodulation – Acetylene reduction assay (ARA)

Biological N₂ fixation is a process with a high energy demand and a high consumption of carbohydrates by

the nodules. In some growth stages this process consumes a substantial fraction (30%–50%) of the plant's net daily photosynthates (Minchin et al. 1981). It follows, therefore, that the efficiency of energy use by the nodules may have considerable implications for seed production.

A minimum theoretical cost of 28 ATP equivalents has been estimated for each molecule of N₂ fixed, assuming the allocation of 25% of the electrons to the obligatory ATP-dependent H₂ evolution (Salsac et al. 1984; Neves and Hungria 1987). Part of the energy lost with the H₂ evolution can be recovered by the action of an uptake hydrogenase system, but in many symbiotic systems more than 50% of total electron flux to nitrogenase is lost through H₂ evolution (Schubert and Evans 1976).

Initially it was believed that the H₂ metabolism was controlled only by the *Rhizobium* strain (Dixon 1967; Carter et al. 1978). Dixon (1972) was the first to suggest that H₂ evolution could also be controlled by the host plant. This was later confirmed in other experiments (Gibson et al. 1981; Lopez et al. 1983; Hungria and Neves 1987). However, the true importance of H₂ metabolism in nodules for the plant growth is still under debate. It was proposed that the relatively small energy saving promoted by the uptake hydrogenase could explain the absence of differences in total N accumulated by Hup⁺ and Hup⁻ strains of *Trifolium* (Gibson et al. 1981), and could also help to explain the absence of differences between plants inoculated with Hup⁺ and Hup⁻ strains or strains with different relative efficiencies (Nelson and Child 1981; Nelson 1983). Meanwhile, many experiments have shown differences in plant dry weight, percentage of N or total N in plants, seed yield, and N-harvest index of either greenhouse or fieldgrown plants, showing differences in H₂ uptake capacity (Schubert et al. 1978; Zablutowicz et al. 1980; Hanus et al. 1981; Hungria

and Neves 1987). Furthermore, some studies have indicated the possibility that H_2 metabolism in the nodules can be linked to other genetic characteristics of metabolic pathways, such as nodulation capacity (Brewin et al. 1980), nodule efficiency (Hungria and Neves 1987), CO_2 fixation in nodules (Arima 1981) and ureide metabolism (Hungria and Neves 1987). These relationships may also help to explain the better performance of low H_2 -evolving strains.

In the present study, we investigated the role of bean cultivar and *Rhizobium* strain on the H_2 evolution by the nodules, and the cultivar's relationship to plant growth and N accumulation in tissues. Further, we evaluated the experimental cost of N_2 fixation in different symbiotic combinations by measuring the CO_2 evolution of detached nodules or nodulated roots.

Materials and methods

Plant materials and growth conditions. The experiment was performed in the greenhouse at Centro de Energia Nuclear na Agricultura, Piracicaba, São Paulo. The *Rhizobium leguminosarum* biovar *phaseoli* strains C-05 (CENA, Piracicaba), C-40 (= CIAT 255; CIAT, Colombia) and C-89 (= CIAT 57, CIAT, Colombia) were grown in yeast mannitol agar medium (Vincent 1970) for four days at 28°C with agitation. Seeds of *Phaseolus vulgaris* cvs. Venezuela-350, Aroana, Moruna, and Carioca were surface-sterilized with 0.2% $HgCl_2$ (Vincent 1970), treated with 1 ml inoculant (approximately 10^8 cells ml^{-1}) for each 15 seeds, and then incubated for 1 h. Four seeds were planted per Leonard jar (Vincent 1970) containing washed and sterilized sand and vermiculite (1:2, V:V mixture). Eight days after emergence the plants were thinned to two plants per pot.

Every 5 days, the plants were fertilized with an N-free nutrient solution as described by Hungria and Neves (1987). Distilled water was supplied daily and mean temperatures during the experiment were 27.8°C/21.1°C (day/night).

The experiment was laid out in a randomized complete block design with 6 replicates, 12 treatments and 4 harvests, totaling 288 pots. Harvests were taken 25 (vegetative stage), 40 (beginning of pod formation) and 55 (mid-pod-fill) days after emergence.

Analysis. Nitrogenase activity (acetylene reduction assay) was estimated using the excised roots of six plants (three pots) detached at the cotyledonary node and sealed with "Serum caps" in 500- to 1000-ml jars (occupying a maximum of 20% of the enclosed volume) (Mague and Burris 1972), and incubated under 13% acetylene for 30 min. Ethylene was analyzed with a Beckman GC-65 gas chromatograph equipped with an H_2 flame ionization detector and a 50-cm glass column with 0.64-cm external diameter filled with Poropak N (100–200 mesh) operated at 50°C with a carrier-gas (N_2) flow rate of 40 $ml\ min^{-1}$. Subsequent tests with these cultivar/strain combinations showed that mean acetylene reduction rates (over 30 min) with detached roots in a closed system were not significantly different from those obtained with whole plants in a continuous flux system.

The CO_2 evolution from nodulated roots was evaluated using the other three pots (six plants). Detached roots were incubated for 10 min in 500–1000 ml bottles and samples were taken for analysis of the CO_2 evolution by nodulated roots. After this incubation the nodules were detached from the roots and incubated for 20 min in

70-ml bottles hermetically sealed (not occupying more than 20% of total volume), for the determination of H_2 and CO_2 evolution. The samples were analyzed with a Beckman GC-65 gas chromatograph with a thermal conductivity detector operated at 40°C and a stainless steel column (0.32 cm external diameter) filled with Poropak Q (80–100 mesh) using either Ar (evaluation of H_2) or H_2 (evaluation of CO_2) as the carrier gas, both at a rate of 20 $ml\ min^{-1}$.

The relative efficiency of the electrons used by the nitrogenase was determined according to the equation of Schubert and Evans (1976):

$$\text{Relative efficiency} = 1 - \frac{H_2 \text{ evolved (air)}}{C_2 H_2 \text{ reduced}}$$

Dry weights of stems, leaves, roots, pods, and nodules were recorded, and the N content in plant tissues was determined by a semimicro Kjeldahl method as reported by Sarruge and Haag (1974).

Results

The bean cultivar, *Rhizobium* strain, had an effect and there was an interaction effect between both symbiotic partners on the acetylene reduction rates, H_2 evolution and relative efficiency of nitrogenase during the growth cycle. The cultivar Carioca showed the highest rates of acetylene reduction from the first harvest onwards (Fig. 1a). This cultivar also presented the highest RE at all harvests (Fig. 2).

At the first harvest (25 days after emergence) the plants inoculated with strain C-40 presented the highest rates of acetylene reduction activity (Fig. 1b) and relative nitrogenase efficiency (Fig. 2) but, after this, the best symbiotic combinations were obtained with plants inoculated with strain C-05 (Figs. 1b and 2).

The interaction effect between cultivars and strains allowed them both to better express their potential for N_2 fixation. Consequently, at 40 days after emergence, the best symbiotic combination, Carioca × C-05, presented an acetylene reduction rate three times that of the poorest combination, Venezuela-350 × C-89 (data statistically different, but not shown) and also lost 20% less electrons through H_2 evolution by the nodules (Fig. 2).

After the 40-day period, there was a drastic drop in acetylene reduction activity for all symbiotic combinations. The relative nitrogenase efficiency also decreased after 40 days (Fig. 2), but even then the symbiotic combination of the cultivar Carioca inoculated with strain C-05 continued to show the highest relative efficiency (Fig. 2).

At 40 days after emergence, when nitrogenase activity reached its peak, the respiration rates of the nodulated roots and nodules were also higher. Nodules represented 17%–26% of total root respiration 25 days after emergence, and 22%–30% at 40 days after emergence (Table 1).

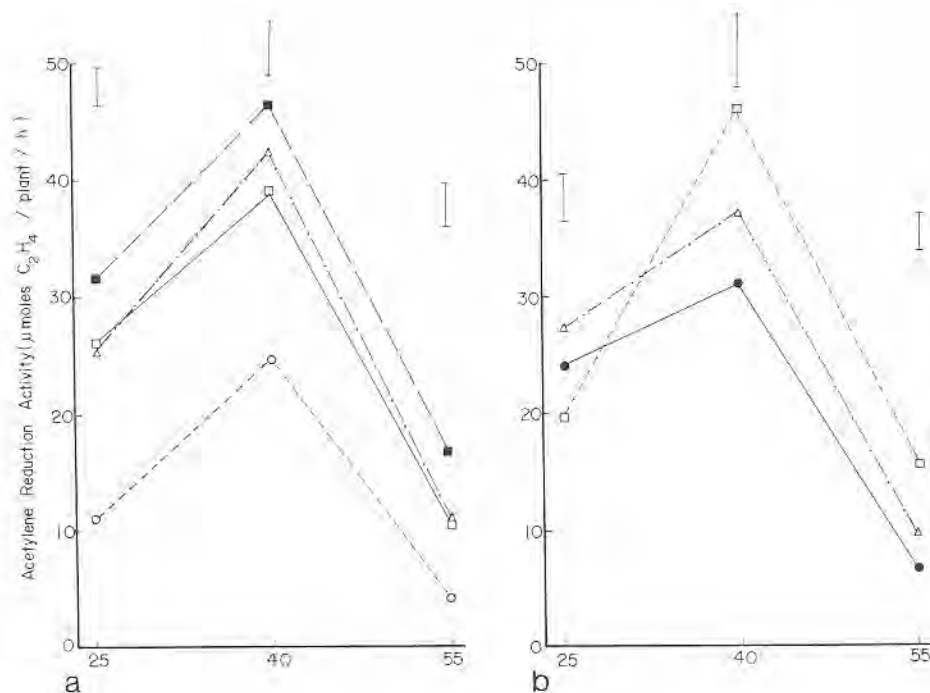


Fig. 1. Effect of a bean (*Phaseolus vulgaris*) cultivar, and b *Rhizobium* strain on nitrogenase activity 25, 40, and 55 days after emergence. Vertical bars denote LSD between cultivars and strains at $P = 0.05$ (Tukey's test). a ■—■ Carioca; Δ — Δ Moruna; \square — \square Aroana; \circ — \circ Venezuela-350. b \square — \square C-05; \bullet — \bullet C-89 (= CIAT 57); Δ — Δ C-40 (= CIAT 255)

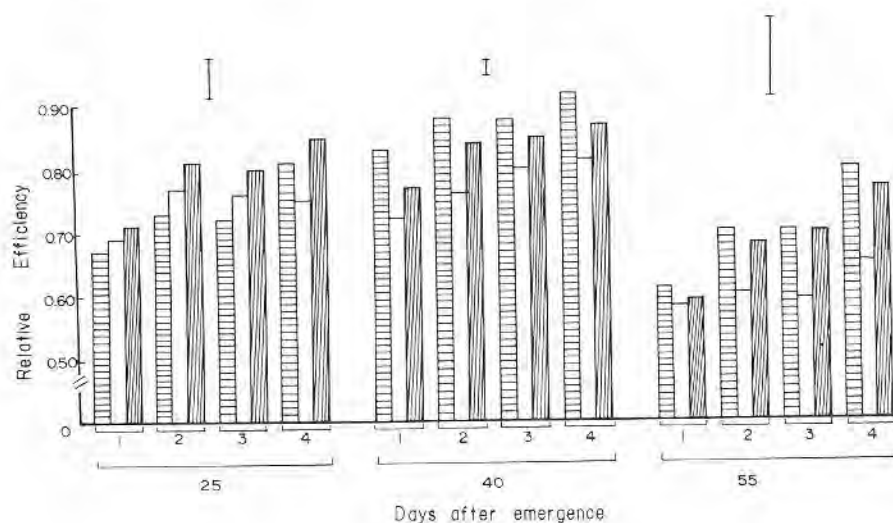


Fig. 2. Changes in the relative efficiency of nitrogenase during the growth of four bean cultivars inoculated with three *Rhizobium* strains. Values are means of three replicates and vertical bars denote LSD between treatment means at $P = 0.05$ (Tukey's test). Bean cultivar: 1: Venezuela-350; 2: Aroana; 3: Moruna; 4: Carioca. *Rhizobium* strain: \square , C-05; \square , C-89 (= CIAT 57); \square , C-40 (= CIAT 255)

The cultivar Carioca produced the highest dry weight of shoots, roots, and nodules from the first harvest onwards (Table 2) and, at 55 days after emergence, the pod yield of this cultivar was respectively 18%, 23% and 230% higher than cultivars Aroana, Moruna, and Venezuela-350. The same pattern was observed in relation to pod N content (Table 3). Plants inoculated with strain C-05 accumulated 59% and 31% more N in pods than plants inoculated with strains C-89 and C-40, respectively (Table 3). The *Rhizobium* strain affected nodule mass, and plants inoculated with C-89 presented higher nodulation (Table 2). Strain C-89 in combination with the cultivar Ven-

zuela-350 accumulated, at mid-pod-fill stage (55 days after emergence), only 10% of the pod N content of the cultivar Carioca inoculated with strain C-05, and also presented a N-harvest index of 0.15, while in the best symbiotic combination this value was 0.43 (Table 3). Differences in the N-harvest index were related to plant cultivar effects and to an interaction between plant and strain, and no effect could be attributed to the *Rhizobium* strain alone (data analyzed statistically, but not shown).

The maximum rates of N assimilation were recorded for the Carioca \times C-05 combination ($11.93 \text{ mg N plant}^{-1} \text{ day}^{-1}$), between 40 and 55 days after emer-

Table 1. Respiration rates ($\text{mg CO}_2 \text{ plant}^{-1} \text{ h}^{-1}$) of nodulated roots and nodules of four bean (*Phaseolus vulgaris*) cultivars inoculated with three *Rhizobium* strains. Means of three replicates^a

Bean cultivar	<i>Rhizobium</i> strain	Days after emergence					
		25		40		55	
		Roots	Nodules	Roots	Nodules	Roots	Nodules
Venezuela 350	C-05	1.81 c	0.32 c	5.95 a	1.31 ab	1.95 cd	0.49 cd
	C-89	2.43 bc	0.63 abc	4.16 abc	1.25 ab	0.62 d	0.16 d
	C-40	2.38 bc	0.40 bc	4.59 ab	1.19 ab	2.21 cd	0.55 cd
Aroana	C-05	3.03 abc	0.57 abc	6.42 a	1.61 a	5.82 bc	1.10 bc
	C-89	3.57 ab	0.78 a	2.01 c	0.60 b	4.82 bc	1.06 bc
	C-40	3.52 ab	0.60 abc	2.52 bc	0.63 b	4.25 bcd	0.89 bc
Moruna	C-05	2.85 bc	0.54 abc	6.75 a	1.69 a	5.02 bc	1.00 bc
	C-89	3.54 ab	0.72 ab	2.24 bc	0.63 b	3.26 bcd	0.91 bc
	C-40	3.48 ab	0.63 abc	3.60 abc	0.90 ab	3.93 bcd	0.79 c
Carioca	C-05	3.58 ab	0.72 ab	6.01 a	1.32 ab	11.05 a	2.21 a
	C-89	3.49 ab	0.80 a	2.59 bc	0.70 b	4.63 bc	1.01 bc
	C-40	4.35 a	0.83 a	2.49 bc	0.55 b	7.27 ab	1.45 b

^a Values followed by the same letter are not significantly different at $P = 0.05$ (Tukey's test) for each vertical line

gence while at the same time the rate for Venezuela-350 \times C-89 was only $0.55 \text{ mg N plant}^{-1} \text{ day}^{-1}$ (Table 3).

Rates of C respired per N fixed are shown in Table 4. The lower rates were recorded for strain C-05 and the cultivar Carioca, for both nodulated roots or detached nodules, indicating a higher energy efficiency for N_2 fixation.

Discussion

Commercial bean cultivars used in this experiment showed large differences in relation to nitrogenase activity, relative efficiency and plant N content, demonstrating the potential for enhancing N_2 fixation in this crop, as reported earlier (Ruschel et al. 1979; Westerman and Kolar 1978; Hungria and Neves 1987). The best performance of the cultivar Carioca was related to its growth habit. It belongs to group III (indeterminate with a tendency to climb) while the other ones belong to group II (bush type). According to Graham (1981), indeterminate and climbing bean cultivars transfer more soluble carbohydrates to the nodules, which explains the higher N_2 -fixation rates.

Although the *Rhizobium* strains used were commercially recommended and nodulated very well, the higher nodule mass produced by C-89 did not correspond to a higher accumulation of N in tissues. This observation, added to previous reports (Saito et al. 1980a, Hungria and Neves 1987) shows that nodule efficiency is an important parameter to be considered in strain selection, since the C sources obtained from the plant's carbohydrates for use in nodule formation and

function must be used efficiently for nodule formation and N_2 -fixation.

The best symbiotic combinations were characterized by a higher relative nitrogenase efficiency, confirming other results reported for beans (Hungria and Neves 1987). However, relative efficiency values at 25–55 days after emergence varied between 0.53 and 0.87, indicating that in *Phaseolus vulgaris*-*Rhizobium leguminosarum* bv. *phaseoli* combinations there is a waste of energy through H_2 evolution by the nodules, as observed by Saito et al. (1980b), Pacovsky et al. (1984), and Hungria and Neves (1987). The effects of strain and host plant, and the interaction between both on relative nitrogenase efficiency show that the control of electron allocation to nitrogenase, or the control of uptake hydrogenase, is much more complicated than first suggested (Dixon 1967; Carter et al. 1978), and that both symbiotic partners are involved.

The best symbiotic combination, Carioca \times C-05, presented a mean relative efficiency of 0.80 during the plant growth cycle, while in the poorest symbiotic combination, Venezuela-350 \times C-89, this value was 0.61. However, in energetic terms, the difference in ATP saving between these two symbiotic combinations due to a higher relative efficiency is insufficient to explain the increase of 244% in total plant N content. One possibility is that other important factors associated with the higher N_2 fixation are genetically linked to the genes responsible for the H_2 metabolism. These factors could include, among others, a higher production of ureides (Hungria and Neves 1987; Neves and Hungria 1987) or a higher CO_2 fixation by the nodules due to a more active phosphoenolpyruvate carboxylase (Arima 1981).

Table 2. Dry weight of shoots (stems+leaves), roots, nodules, and pods of four bean (*Phaseolus vulgaris*) cultivars inoculated with three *Rhizobium* strains. Harvests taken 25, 40, and 55 days after emergence (DAE)

Bean cultivar	<i>Rhizobium</i> strain	Dry weight (g plant ⁻¹) ^a				
		Shoots	Roots	Nodules	Pods	Whole plant
25 DAE						
Venezuela-350	C-05	0.97 g	0.57 a	0.102 d		1.65 f
	C-89	1.03 g	0.60 a	0.121 c		1.75 f
	C-40	1.19 fg	0.62 a	0.091 d		1.91 f
Aroana	C-05	1.66 de	0.67 a	0.096 d		2.43 de
	C-89	1.91 cd	0.66 a	0.103 d		2.68 cde
	C-40	1.94 cd	0.66 a	0.101 d		2.71 cde
Moruna	C-05	1.54 ef	0.65 a	0.125 c		2.32 e
	C-89	1.99 cd	0.65 a	0.120 c		2.77 cd
	C-40	2.11 bc	0.68 a	0.120 c		2.92 bc
Carioca	C-05	2.07 bc	0.70 a	0.130 bc		2.91 bc
	C-89	2.42 b	0.64 a	0.148 a		3.21 b
	C-40	3.39 a	0.64 a	0.143 ab		4.19 a
	LSD (0.05) ^b	0.36	NS	0.010		0.39
	LSD (0.05) ^c	0.21	0.05	0.010		0.22
40 DAE						
Venezuela-350	C-05	3.45 bc	1.45 def	0.187 e		5.09 cd
	C-89	3.32 c	1.33 f	0.250 a		4.91 d
	C-40	3.33 c	1.41 ef	0.228 b		4.97 d
Aroana	C-05	4.43 a	1.82 a	0.189 e		8.16 a
	C-89	3.17 c	1.46 de	0.210 cd		4.84 d
	C-40	3.41 bc	1.66 dc	0.195 de		5.27 cd
Moruna	C-05	4.43 a	1.58 c	0.196 de		7.98 a
	C-89	3.21 c	1.55 cd	0.235 ab		5.00 d
	C-40	3.77 b	1.55 cd	0.244 bc		5.55 c
Carioca	C-05	4.48 a	1.82 a	0.219 bc		6.53 b
	C-89	3.34 c	1.46 de	0.250 a		5.05 d
	C-40	4.15 a	1.73 ab	0.234 b		6.12 b
	LSD (0.05) ^b	NS	0.21	0.010		0.46
	LSD (0.05) ^c	0.21	0.14	0.010		0.26
55 DAE						
Venezuela-350	C-05	4.20 cdef	1.52 a	0.230 d	1.12 g	7.08 e
	C-89	3.52 f	1.50 a	0.251 bc	0.74 h	6.02 f
	C-40	3.93 def	1.57 a	0.252 ab	0.95 gh	6.71 ef
Aroana	C-05	5.98 a	1.81 a	0.231 c	2.95 bc	10.98 ab
	C-89	4.14 def	1.64 a	0.270 ab	2.34 f	8.39 d
	C-40	4.39 cde	1.64 a	0.232 cd	2.57 de	8.84 d
Moruna	C-05	5.68 ab	1.79 a	0.226 d	2.75 cd	10.46 bc
	C-89	3.95 def	1.64 a	0.252 ab	2.32 f	8.17 d
	C-40	4.65 cd	1.67 a	0.230 d	2.46 ef	9.02 d
Carioca	C-05	5.88 a	1.82 a	0.261 ab	3.61 a	11.59 a
	C-89	3.85 ef	1.62 a	0.271 a	2.52 ef	8.27 d
	C-40	4.94 bc	1.71 a	0.259 ab	3.15 ab	10.06 c
	LSD (0.05) ^b	0.75	NS	0.020	0.21	0.86
	LSD (0.05) ^c	0.43	0.13	0.012	0.12	0.45

^a Values followed by the same letter are not significantly different at $P = 0.05$ (Tukey's test) for the interaction cultivar \times strain in each harvest. Means of five replicates

^b LSD (0.05) for the means between cultivars; NS, non significant

^c LSD (0.05) for the means between strains

On average, for all symbiotic systems during the whole growth cycle the nodules were responsible for 23% of the total respiration of the nodulated roots.

This evaluation of the experimental cost of N₂ fixation is subject to many criticisms (Minchin et al. 1981). In the present experiment, the lower ratio of mg

Table 3. Total N of shoots (stems+leaves), roots, nodules, and pods of four bean (*Phaseolus vulgaris*) cultivars inoculated with three *Rhizobium* strains. Harvests taken 25, 40, and 55 days after emergence (DAE)

Bean cultivar	<i>Rhizobium</i> strain	Total N (mg plant ⁻¹) ^a				
		Shoots	Roots	Nodules	Pods	Whole plant
25 DAE						
Venezuela-350	C-05	26.51 e*	7.54 f	4.56 d		38.62 h
	C-89	33.26 e	11.50 e	4.59 d		49.36 g
	C-40	38.14 e	14.16 cd	3.75 e		56.06 f
Aroana	C-05	61.47 cd	12.73 de	5.03 d		79.23 d
	C-89	69.97 bc	13.88 d	5.18 cd		89.04 e
	C-40	76.88 b	16.41 ab	4.96 d		98.25 b
Moruna	C-05	55.00 d	11.45 e	6.87 a		73.34 c
	C-89	69.32 bc	13.16 de	5.88 bc		88.37 c
	C-40	75.69 b	17.27 a	5.99 b		98.96 b
Carioca	C-05	75.26 b	12.79 b	6.90 a		94.96 b
	C-89	64.60 bcd	14.36 bcd	6.78 a		85.74 c
	C-40	104.92 a	16.36 abc	6.87 a		128.15 a
	LSD ^b	13.57	2.27	0.78		4.66
	LSD ^c	7.83	1.31	0.45		7.65
40 DAE						
Venezuela-350	C-05	96.05 de	27.71 e	8.17 fg		131.94 ef
	C-89	77.62 ef	20.38 f	7.45 g		105.45 g
	C-40	86.20 ef	22.97 f	8.90 ef		118.90 efg
Aroana	C-05	126.59 bc	47.18 a	8.43 e		183.22 b
	C-89	74.44 f	30.45 de	9.19 e		114.10 fg
	C-40	90.68 def	37.42 c	7.98 g		136.08 de
Moruna	C-05	133.68 ab	42.40 b	10.35 cd		186.43 b
	C-89	84.05 ef	26.03 e	9.71 de		119.80 efg
	C-40	107.98 cd	36.01 c	9.72 de		153.73 cd
Carioca	C-05	148.83 a	51.17 a	11.75 a		211.77 a
	C-89	86.86 ef	27.56 e	10.66 bc		125.09 ef
	C-40	121.69 bc	34.01 cd	11.45 ab		167.19 bc
	LSD ^b	20.79	4.48	0.88		20.21
	LSD ^c	12.01	2.58	0.50		11.67
55 DAE						
Venezuela-350	C-05	97.23 cd	20.82 de	9.73 cd	31.61 f	159.42 fg
	C-89	71.86 e	17.39 e	7.57 e	16.82 g	113.66 h
	C-40	93.78 cd	19.98 de	9.36 d	26.21 f	149.33 g
Aroana	C-05	138.76 b	34.20 a	10.93 bc	96.37 c	280.73 b
	C-89	80.97 cde	19.95 de	10.60 bc	72.47 e	184.00 de
	C-40	89.80 cde	23.44 cd	9.37 d	80.13 de	202.75 cd
Moruna	C-05	137.49 b	34.73 a	11.02 b	94.01 c	277.26 b
	C-89	70.42 e	21.41 d	10.10 bcd	74.99 de	176.93 ef
	C-40	97.80 c	25.78 c	11.00 b	81.44 de	216.03 c
Carioca	C-05	169.41 a	36.39 a	15.13 a	169.83 a	390.77 a
	C-89	78.28 de	22.23 cd	10.99 b	82.87 d	194.38 cde
	C-40	124.72 b	29.67 b	14.77 a	110.19 b	279.38 b
	LSD ^b	19.42	3.70	1.19	9.56	23.09
	LSD ^c	11.21	2.13	0.69	5.52	13.33

See notes to Table 2

C lost as CO₂ per mg N fixed, which was 5.7 for nodulated roots and 1.2 for detached nodules, corresponded to the best symbiotic combination that of Carioca×C-05, and the lower evolution of H₂ by this system probably had a substantial effect on the higher

efficiency of nodule respiration. Mahon (1979), working with *Phaseolus vulgaris* beans, reported a ratio of 6.1 for nodulated roots, and use of detached nodules of cowpea gave ratios between 1.1 (Atkins et al. 1978) and 2.4 (Neves et al. 1981).

Table 4. Ratio of C lost (as CO₂) for nodulated roots or nodules to N₂ fixed during the growth of four bean (*Phaseolus vulgaris*) cultivars inoculated with three *Rhizobium* strains. Means of three replicates^a

Bean cultivar	<i>Rhizobium</i> strain	Days after emergence							
		0-25		26-40		41-55		Mean	
		Roots	Nodules	Roots	Nodules	Roots	Nodules	Roots	Nodules
Venezuela-350	C-05	7.6a	1.4bc	6.2de	1.4de	6.9ab	1.7ab	6.9	1.5
	C-89	8.0a	2.0a	7.2b	2.2ab	7.3a	1.9a	7.5	2.0
	C-40	6.9b	1.2cd	7.2b	1.9bc	6.9ab	1.7ab	7.0	1.6
Aroana	C-05	6.2cde	1.2cd	6.0de	1.5d	5.8f	1.3cd	6.0	1.3
	C-89	6.5bc	1.4bc	7.8a	2.3a	6.7b	1.5bc	7.0	1.7
	C-40	5.8def	1.0d	6.5cd	1.6cd	6.2def	1.3cd	6.2	1.3
Moruna	C-05	6.3cd	1.2cd	5.8e	1.4de	5.9ef	1.1d	6.0	1.2
	C-89	6.5bc	1.4bc	6.9bc	1.9bc	6.7b	1.2cd	6.7	1.5
	C-40	5.7ef	1.0d	6.4cd	1.6cd	6.5bcd	1.2cd	6.2	1.9
Carioca	C-05	6.1cde	1.2cd	5.0f	1.1e	6.0ef	1.2cd	5.7	1.2
	C-89	6.6bc	1.5b	6.5cd	1.7cd	6.5bcd	1.4bcd	6.5	1.5
	C-40	5.5f	1.0d	6.2de	1.4de	6.3cde	1.3cd	6.0	1.2

^a Values followed by the same letter are not significantly different at $P = 0.05$ (Tukey's test) for each vertical line

In the present experiment we tried to measure some physiological parameters of N₂ fixation in the symbiosis *Phaseolus vulgaris*-*Rhizobium leguminosarum* biovar *phaseoli*. We conclude that although all combinations of cultivars and strains used were well nodulated there were big differences in nodule efficiency and also in H₂ evolution. Due to these factors, and possibly others not evaluated, there were huge differences in total N accumulated in the plant tissues. The metabolic pathways leading to large difference in nodule efficiency between different symbiotic systems therefore require further investigation.

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