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Partitioning of nitrogen from biological fixation and fertilizer in *Phaseolus vulgaris*

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Nitrogen uptake, distribution and remobilization in the vegetative and reproductive parts of the plant were studied in bean (*Phaseolus vulgaris* L.) cultivars Negro Argel and Rio Tibagi inoculated with either *Rhizobium* strain C05 or 127 K-17. Greenhouse grown plants were supplied with 2.5 mg N (plant)$^{-1}$ day^{-1} as KNO_3 or $K^{15}NO_3$ and the relative contribution to total plant nitrogen of mineral and symbiotically fixed nitrogen was determined. Control plants included those entirely dependent on fixed nitrogen as well as uninoculated plants supplied with 10 mg N (plant)$^{-1}$ day^{-1}. No differences were observed between inoculated treatments in total nitrate reductase activity and in the amount of mineral nitrogen absorbed, but there were considerable differences in the contribution of fixed nitrogen. Nitrogen fixation supplied from 58 to 72% of the total nitrogen assimilated during the bean growth cycle and the symbiotic combinations fixed most of their nitrogen (66 to 78% of total nitrogen) after flowering. Maximum uptake of mineral nitrogen was in the 15-day-period between flowering and mid-podfill (47 to 58% of total mineral nitrogen). Nitrogen partitioning varied with *Rhizobium* strains, and inoculation with strain C05 increased the nitrogen harvest index of both cultivars. Applied mineral nitrogen had a variable effect and in cv. Negro Argel was more beneficial to vegetative growth, resulting in smaller nitrogen harvest indices. Seed yield was not increased by heavy nitrogen fertilization. In contrast, cv. Rio Tibagi always benefited from nitrogen applications. Among the various nitrogen sources supplying the grain, the most important one was the fixed nitrogen translocated directly from nodules or after a rapid transfer through leaves, representing from 60 to 64% of the total nitrogen incorporated into the seeds.

Additional key words – Bean, nitrate reductase, nitrogen assimilation, nitrogen transport, nitrogenase, nodulation, *Rhizobium*, ureides.

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Introduction

Plant breeding programs aimed at the improvement of seed quality and yield of grain legumes often require detailed information about the distribution and remobilization of nitrogen through vegetative and reproductive parts of the plant, since these factors can change seed yield and nitrogen harvest indices. Consequently, studies on the distribution of nitrogen from soil and fertilizers, and nitrogen from fixation in legumes permit not only the selection of the most efficient symbiotic systems, but also the most effective time to apply mineral nitrogen (Eaglesham et al. 1977, Pate and Herridge 1978). Furthermore, the mobilization can differ with plant genotype (Israel 1981, Ruschel et al. 1982) and *Rhizobium* strain (Israel 1981, Morris and Weaver 1983).

Quantitative information on nitrogen assimilation, distribution and redistribution in nodulated legumes is available for peas (Pate and Flinn 1973), soybeans (Sinclair et al. 1980), white lupin (Pate and Herridge 1978, Pate et al. 1979) and cowpeas (Herridge and Pate 1977, Minchin et al. 1981, Neves et al. 1981) and also on the nitrogen economy of developing fruits of cowpea (Peo-

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ples et al. 1983). However, limited information is available on tropically adapted *Phaseolus* beans, which, have a short nitrogen assimilation period due to their life cycle (70 days for most commercial cultivars). This characteristic has often raised doubts on the adequacy of nitrogen fixation in providing nitrogen for large seed yields of bean plants. Furthermore, breeding programs are carried out with high nitrogen fertilizer levels, creating a selection pressure in favour of cultivars unable to use their full potential for nitrogen fixation (Döbereiner and Duque 1980).

The present investigation reports on nitrogen cycling in the vegetative plant components from the pre- to the post-flowering stage and on the relative contribution to grain yield of symbiotically fixed nitrogen in comparison with inorganic sources of nitrogen to grain yield.

Abbreviations – DAE, days after emergence; Ndfa, nitrogen derived from atmosphere; Ndff, nitrogen derived from fertilizer; NR, nitrate reductase.

Materials and methods

Plants were grown in a greenhouse at EMBRAPA-UAPNPBS, Km 47, Rio de Janeiro, from September to December, 1983. Metal pots (6 l) were filled with a mixture of washed sand and vermiculite (1:2; v:v) and sterilized at 120°C for 36 h. The bean (*Phaseolus vulgaris* L.) seeds of cvs Negro Argel and Rio Tibagi (EMBRAPA-CNPAF, Goiânia) were surface-sterilized with 0.2% $HgCl_2$ (Vincent 1970) and separately inoculated with 1 ml of inoculant (10^8 cells ml^{-1}) for each of 15 seeds. The inoculants were *Rhizobium leguminosarum* biovar. *phaseoli*, strain C05 (isolated in Piracicaba, São Paulo) or strain 127 K-17 (Nitragin Co., USA) individually grown in YMA medium (Vincent 1970) for 4 days at 28°C with agitation. Each pot received 5 inoculated seeds and was covered with a layer of approximately 3 cm of sterile sand.

Nitrogenase, nitrate reductase and total N that accumulated in plants were investigated in inoculated plants receiving 0 or 2.5 mg N $(plant^{-1})$ day^{-1} and in uninoculated plants receiving 10.0 mg N $(plant^{-1})$ day^{-1}, with four replicates in each harvest. The harvests were at 30, 35, 50 and 70 DAE for nitrogenase activity, at 25, 30, 40 and 55 DAE for nitrate reductase and at 70 DAE for total N.

Plants were thinned to two per pot 4 DAE and they received 200 ml (pot^{-1}) of nutrient solution applied every 5 days. The nutrient solution contained 0.725 m*M* NaH_2PO_4, 0.725 m*M* KH_2PO_4, 3.1 m*M* $MgSO_4 \cdot 7H_2O$, 2.9 m*M* $CaSO_4 \cdot 2H_2O$, 1.5 m*M* K_2SO_4, 1 μ*M* $MnSO_4 \cdot 4H_2O$, 0.1 μ*M* $CuSO_4 \cdot 5H_2O$, 0.1 μ*M* $ZnSO_4 \cdot 7H_2O$, 5.0 μ*M* H_3BO_3, 10.0 μM NaCl, 0.5 μ*M* $NaMoO_4 \cdot 2H_2O$, 0.02 μ*M* $CoSO_4 \cdot 6H_2O$ and 5 μ*M* FeEDTA, pH 6.0 to 6.2. Distilled water was supplied daily based on pot weight.

Nitrogenase activity was determined using the acetylene reduction technique in a continuous flow system (Minchin et al. 1983) with 12% acetylene in air at a rate of 100 ml min^{-1}. Samples were taken after 4 min of incubation. Acetylene and ethylene were analysed using a Perkin-Elmer F-11 gas chromatograph equipped with a hydrogen flame ionization detector and a 50 cm stainless steel column (0.32 cm external diameter) filled with Poropak N(80–100 mesh) operated at 40°C with a carrier gas (N_2) flow rate of 40 ml min^{-1}.

Nitrate reductase (NR) activity was evaluated according to Andrews et al. (1984). The NR activity measured in leaves (discs of 2 by 2 mm, weighing 0.2 to 0.3 g), stems (0.3 to 0.4 g), roots (0.5 to 0.7 g) and nodules (0.1 to 0.2 g of nodule fresh weight) and the total activity was expressed relative to the total plant fresh weight.

Xylem sap was collected at 35 DAE. Plants were cut off at the cotyledonary node and the cut stem was washed and dried with tissue paper. The exuded sap was collected in calibrated microcapillaries (20 and 50 μl) for 15 min so that the exudation rate could be calculated. The sap was stored at −20°C until analysis. Nitrogen compounds were analysed colorimetrically after Hungria et al. (1985).

The partitioning of nitrogen was studied in plants receiving 2.5 mg N $(plant)^{-1}$ day^{-1} (1.25 mg in the first 10 days) as KNO_3 or $K^{15}NO_3$ (0.398 atom % ^{15}N excess). Plants received ^{15}N up to first flowering, 35 DAE (and then ^{14}N to maturity), ^{15}N up to mid-podfill stage (and then ^{14}N to maturity) or ^{15}N throughout both vegetative and reproductive periods (a crop duration of 70 days). Harvests were performed at flowering, mid-podfill stage or maturity, with four repetitions for each treatment. Senesced leaves were collected as necessary. Plants were separated into their various vegetative and reproductive parts and dry weights recorded. All components of plants were analysed for nitrogen content (including nitrate) using the method of Liao (1981). The labelled plants were analysed for ^{15}N enrichment by mass spectrometry (Proksch 1972) using a mass spectrometer Atlas-Varian CH-4. The contribution of nitrogen fixation to the overall nitrogen status of any plant component was calculated according to Eaglesham et al. (1977) as follows:

$$\text{Ndfa in component} = \text{N}_{\text{total}} \text{ in component} - \left(\frac{\text{R in component}}{\text{R in solution}} \times \text{N}_{\text{total}} \text{ in component} \right)$$

and Ndff in component = (N_{total} in component − Ndfa in component) where R = atom% ^{15}N excess.

Results

All plants flowered at 33 to 35 DAE and the final harvest was at 70 DAE. Supplemental nitrogen, equivalent to 2.5 mg N $(plant)^{-1}$ day^{-1}, applied to nodulated plants,

Tab. 1. Seed dry weight and total N, total N in plants and N harvest index in beans nodulated and/or receiving mineral N. Means of 4 replicates, harvest at 70 days after emergence. * Values followed by the same letter are not different at $P = 0.01$ (Tukey's test). ** Numbers in brackets represent the N harvest index for the N derived from fixation.

Cultivar/Strain	Level of mineral N mg N (plant)$^{-1}$ day^{-1}	Seed dry weight g (plant)$^{-1}$	Total N mg N (plant)$^{-1}$		N Harvest index N seed (N plant)$^{-1}$
			Plant	Seed	
Negro Argel/C05	0	5.7c	281cd	197c	0.7a
Negro Argel/127 K-17	0	5.1d	247de	162d	0.6b
Rio Tibagi/C05	0	4.0e	218ef	128f	0.5de
Rio Tibagi/127 K-17	0	2.4f	169f	81g	0.4f
Negro Argel/C05	2.5	6.7a	359ab	237a	0.6b(0.7a)**
Negro Argel/127 K-17	2.5	6.0c	323bc	197c	0.6cd(0.6b)
Rio Tibagi/C05	2.5	5.1d	285cd	162d	0.5e(0.6c)
Rio Tibagi/127 K-17	2.5	5.1d	260de	143e	0.5e(0.6c)
Negro Argel	10.0	6.8a	396a	247a	0.6c
Rio Tibagi	10.0	6.4b	387a	225b	0.5de

stimulated plant growth, nitrogen accumulation and seed yield. However, the nitrogen stimulated a greater response in the less efficient symbioses, especially the total nitrogen in seeds of the least efficient symbiosis (Rio Tibagi/127 K-17), which was increased by 78%. However, in the best symbiotic combination (Negro Argel/C05) the increase due to supplemental nitrogen was only 20% (Tab. 1) There was no synergistic effect between uptake of mineral nitrogen and nitrogen fixation, and mineral nitrogen caused not only a decrease in total nitrogenase activity, but also a delay in the timing of maximum nitrogenase activity in relation to the plants entirely dependent on *Rhizobium* (Fig. 1). During the plant growth cycle, non-nodulated plants supplied with a high level of nitrate [10 mg N(plant)$^{-1}$ day^{-1}] grew more rapidly and had higher seed production and nitrogen accumulation than those totally dependent on nitrogen fixation (Tab. 1). Applying high levels of nitrogen to cv. Negro Argel did not significantly increase seed yield and seed nitrogen in relation to this cultivar inoculated with strain C05 and receiving a low level of nitrogen [2.5 mg N(plant)$^{-1}$ day^{-1}]. Rio Tibagi, however, benefitted from the high nitrogen application even when compared with plants inoculated with strain C05 and receiving low mineral nitrogen.

The partitioning of nitrogen within the plant shoot was modified by the treatments imposed. Inoculation with strain C05 increased the nitrogen harvest index of both cultivars, and cv. Negro Argel had a higher nitrogen harvest index than Rio Tibagi. Except for the poorest symbiotic combination (Rio Tibagi/127 K-17), mineral nitrogen stimulated vegetative growth more than seed yield, as shown by the lower nitrogen harvest indices (Tab. 1). The differential partitioning of mineral

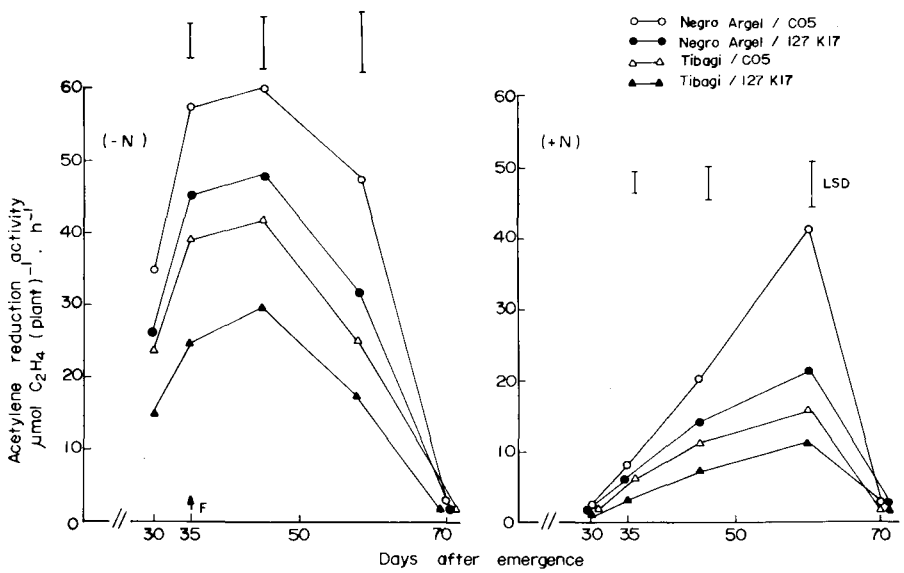

Fig. 1. Acetylene-reduction activity during the growth cycle of bean, cvs Negro Argel and Rio Tibagi, inoculated with *Rhizobium* strains C05 or 127 K-17; + N treatments received 2.5 mg N(plant)$^{-1}$ day^{-1}. Means of 4 replicates; LSD for $P = 0.01$ (Tukey).

Tab. 2. Accumulation and distribution of N derived from fertilizer (Ndff) and from the atmosphere (Ndfa) mg N (plant)$^{-1}$ in bean cv. Negro Argel, receiving 2.5 mg N (plant)$^{-1}$ day^{-1}. Means of 4 replicates. * (A) represents ^{15}N until (F); (B) ^{15}N until (F) and ^{14}N from (F) to (MPF); (C) ^{15}N until (MPF); (D) ^{15}N until (F) and ^{14}N from (F) to (M); (E) ^{15}N until MPF and ^{14}N from (MPF) to (M); (F) ^{15}N until (M).

Plant component	Flowering (F)		Mid podfill (MPF)			Maturity (M)			
	Ndff(A)*	Ndfa	Ndff(B)	Ndff(C)	Ndfa	Ndff(D)	Ndff(E)	Ndff(F)	Ndfa
				Negro Argel/C05					
Root	11.0	6.1	8.4	16.3	11.3	3.7	4.8	6.8	7.2
Stem	8.3	30.4	5.1	13.0	23.8	3.4	5.7	7.6	15.4
Leaf	13.7	52.2	11.8	34.8	49.2	4.2	3.6	4.3	10.2
Senesced leaves	–	–	0.6	1.0	1.5	5.7	16.5	17.2	31.2
Pod	–	–	7.0	24.8	43.9	–	–	–	–
Pod wall	–	–	–	–	–	1.8	4.7	6.8	16.7
Seed	–	–	–	–	–	13.5	54.1	55.9	178.4
Ndff(total plant)	33.0	–	32.9	89.9	–	32.3	89.4	98.6	–
Ndfa(total plant)	–	88.7	–	–	129.7	–	–	–	259.1
				Negro Argel/127 K-17					
Root	11.5	6.2	10.7	16.1	6.8	2.5	4.8	6.2	6.2
Stem	11.5	28.7	10.5	12.7	20.4	2.1	5.9	7.5	15.7
Leaf	14.1	34.8	10.2	30.1	45.3	1.2	3.6	5.2	9.1
Senesced leaves	–	–	1.0	1.4	1.9	8.7	18.7	19.0	35.2
Pod	–	–	5.0	23.1	30.6	–	–	–	–
Pod wall	–	–	–	–	–	2.2	4.9	6.8	15.0
Seed	–	–	–	–	–	20.4	46.2	53.0	144.3
Ndff (total plant)	37.1	–	37.4	83.4	–	37.1	84.1	97.7	–
Ndfa (total plant)	–	69.7	–	–	105.0	–	–	–	225.5

and fixed nitrogen was also illustrated in the bean plants that had two sources of nitrogen, since the nitrogen harvest index for the nitrogen derived from fertilizer was in most cases lower than that for the nitrogen derived from air (Tab. 1).

The contribution of symbiotically fixed or mineral nitrogen to the various parts of the plant and seed development was calculated from plants receiving nutrient solution enriched with ^{15}N [2.5 mg N(plant)$^{-1}$ day^{-1}] at various growth stages.

Tab. 3. Accumulation and distribution of N derived from fertilizer (Ndff) and from the atmosphere (Ndfa) mg N (plant)$^{-1}$ in bean cv. Rio Tibagi, receiving 2.5 mg N (plant)$^{-1}$ day^{-1}. Means of 4 replicates. * (A) represents application of ^{15}N until (F); (B) ^{15}N until (F) and ^{14}N from (F) to (MPF); (C) ^{15}N until (MPF); (D) ^{15}N until (F) and ^{14}N from (F) to (M); (E) ^{15}N until MPF and ^{14}N from (MPF) to (M); (F) ^{15}N until (M).

Plant component	Flowering (F)		Mid podfill (MPF)			Maturity (M)			
	Ndff(A)*	Ndfa	Ndff(B)	Ndff(C)	Ndfa	Ndff(D)	Ndff(E)	Ndff(F)	Ndfa
				Rio Tibagi/C05					
Root	12.4	4.2	10.7	16.8	6.8	2.5	7.6	6.0	7.4
Stem	8.3	13.9	6.5	12.8	17.2	1.5	5.9	6.7	17.6
Leaf	13.6	21.4	10.8	35.8	36.4	0.7	2.0	2.5	3.3
Senesced leaves	–	–	1.0	1.1	1.5	7.8	22.5	23.5	34.4
Pod	–	–	4.0	21.6	20.6	–	–	–	–
Pod wall	–	–	–	–	–	2.2	5.7	8.7	13.9
Seed	–	–	–	–	–	21.2	45.9	59.4	101.5
Ndff (total plant)	34.3	–	33.0	88.1	–	35.8	89.6	106.8	–
Ndfa (total plant)	–	39.5	–	–	82.5	–	–	–	178.1
				Rio Tibagi/127 K-17					
Root	12.8	3.6	12.0	14.9	5.5	2.7	7.6	6.0	4.1
Stem	7.9	12.5	7.5	15.0	15.7	1.9	7.0	5.8	20.8
Leaf	13.6	23.5	10.7	40.9	25.2	0.8	2.4	2.1	4.1
Senesced leaves	–	–	1.3	1.2	1.3	9.1	20.8	29.6	23.1
Pod	–	–	1.8	15.4	9.4	–	–	–	–
Pod wall	–	–	–	–	–	2.2	6.9	8.1	14.1
Seed	–	–	–	–	–	19.2	42.8	56.7	85.1
Ndff (total plant)	34.3	–	32.8	87.4	–	35.9	87.5	108.3	–
Ndfa (total plant)	–	39.6	–	–	57.1	–	–	–	151.3

Seeds of cv. Negro Argel initially contained an average of 6.2 mg N(seed)$^{-1}$, whereas cv. Rio Tibagi had an average of 5.9 mg N(seed)$^{-1}$, which represented less than 4% of total plant N at final harvest. The major source of nitrogen to cv. Negro Argel was symbiotic fixation, supplying 65 to 73% of the total nitrogen assimilated during vegetative growth and 72% of total nitrogen at maturity (calculated from Tabs 2 and 3). For cv. Rio Tibagi, symbiotic fixation represented from 58 to 62% of total nitrogen at maturity.

All four cultivar and strain combinations fixed most of their nitrogen (50 to 62% of the total nitrogen fixed) during the final 20 days of the pod-filling period, with an assimilation rate of 5 and 8 mg N(plant)$^{-1}$ day^{-1} for Rio Tibagi and Negro Argel, respectively. This pattern of nitrogen fixation confirmed the nitrogenase activity estimated by the acetylene reduction assay (Fig. 1).

Although there were large differences in the total nitrogen fixed by the symbiotic systems (columns Ndfa, Tabs 2 and 3), the uptake of mineral nitrogen was very similar for all plants (columns Ndff, Tabs 2 and 3), confirming the nitrate reductase pattern found during plant growth (Fig. 2).

During the vegetative period, plants were supplied with a total of 77.5 mg N in the nutrient solution, but assimilated only an average of 35 mg N(column A, Tabs 2 and 3). Therefore, the efficiency of utilization of the fertilizer was about 45%, whereas the overall efficiency for the whole plant cycle was on the average 62%. Most of the mineral nitrogen was assimilated during the 15 days after flowering, representing 47 to 58% of total mineral nitrogen assimilated.

Mineral nitrogen was extensively used for root growth and represented at flowering 76% of the total nitrogen of the nodulated roots of cv. Rio Tibagi (calculated from Tab. 3). This cultivar also had higher Ndff in the stem, leaf, pod wall and seeds, reflecting its lower nitrogen fixing activity. Higher percentages of Ndff were also recorded for plants inoculated with strain 127 K-17 than for those inoculated with strain C05, reflecting the lower nitrogen fixing activity of the former. The percentage of Ndff for the senesced leaves was on

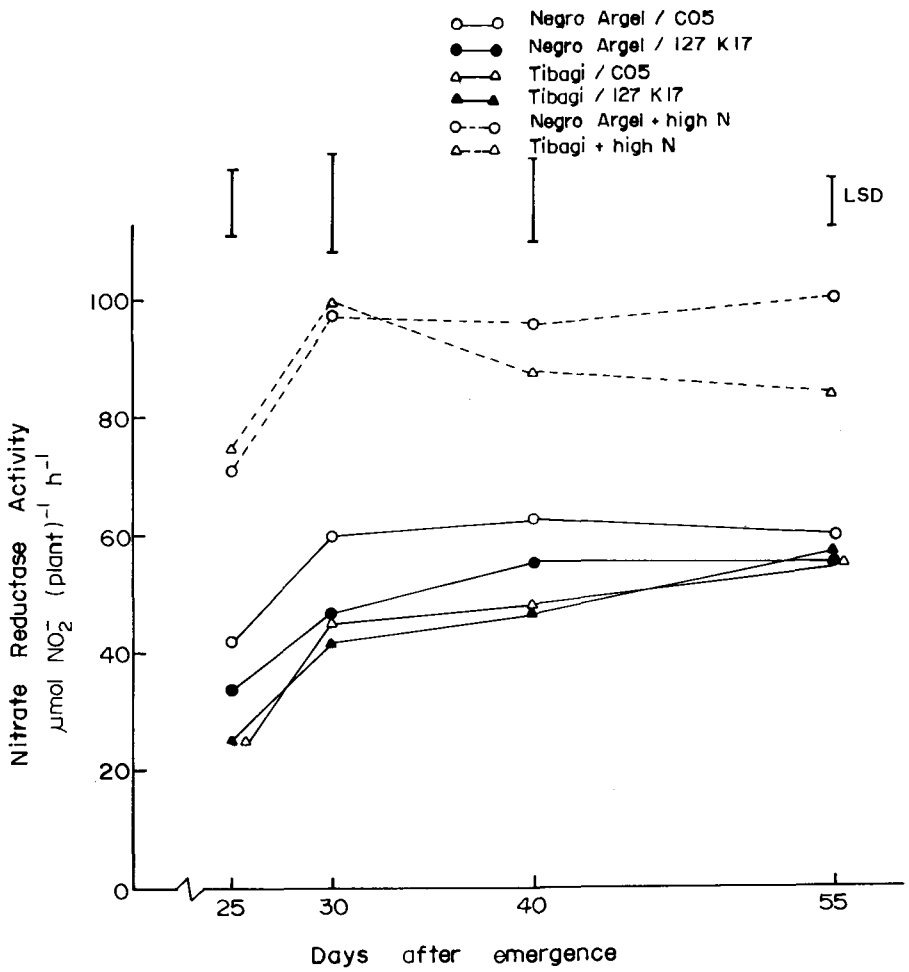

Fig. 2. Nitrate reductase activity during the bean growth cycle. Inoculated plants received 2.5 mg N(plant)$^{-1}$ day^{-1}. Plants receiving 10.0 mg N(plant)$^{-1}$ day^{-1} were not inoculated. Means of 4 replicates; LSD for $P = 0.01$ (Tukey).

the average 47%, contrasting with 37% for the green leaves. This can be seen for all treatments, and the consistent increase in the percentage of Ndff of the senesced leaves indicates that the N derived from the atmosphere was more mobile.

The nitrogen redistribution to the pods was greatly affected by the plant cultivars and *Rhizobium* strains tested. Although the quantity of mineral nitrogen assimilated before flowering was similar for all treatments, the association of Negro Argel/C05 transferred (after 15 days) 21% of this nitrogen to pods, whereas Rio Tibagi/K-17 transferred only 6% [from column Ndff (B), Tabs 2 and 3]. An average of 52% of the pre-flowering mineral nitrogen was redistributed to the fruits, but this nitrogen represented only 6 (Negro Argel/C05) to 10–14% (in the other symbiotic systems) of the nitrogen in fruits at the final harvest (Tabs 2 and 3). An average of 22% of the mineral nitrogen absorbed until flowering was lost in senesced leaves [from column Ndff (D), Tab. 3].

The redistribution to fruits of the mineral nitrogen assimilated during the 15-day-period between flowering and mid-podfill stage was 64% greater for Negro Argel/C05 compared with Rio Tibagi/127 K-17 [from columns Ndff (D) and Ndff (E), Tabs 2 and 3]. Therefore, in this period, the symbiotic system Negro Argel/C05 not only fixed more nitrogen, but also transferred more mineral nitrogen to the seeds. Although the remobilization of the nitrogen to the pods in this period ranged from 44 to 72%, this nitrogen contributed with an average of only 16% to the total nitrogen in grains at maturity. The contribution of the mineral nitrogen assimilated after mid-podfill stage was even smaller, ranging from 1 (Negro Argel/C05) to 10% (Rio Tibagi/127 K-17) of the total nitrogen in seeds [from columns Ndff (E), Ndff (F) and Ndfa, Tabs 2 and 3].

Fixed nitrogen was more readly used for pod growth than was mineral nitrogen. Of the 151 and 259 mg of Ndfa in the contrasting symbiotic systems Rio Tibagi/127 K-17 and Negro Argel/C05, 66 to 75% was transferred to fruits, whereas mineral nitrogen was at most 63% of the total Ndff (Tabs 2 and 3 and Fig. 3). In the two contrasting symbioses, 72 to 75% of the total nitrogen in pods was Ndfa transferred directly to the pods or at least in a rapid transfer through the vegetative parts of the plant, not being detected in the period between the harvests (Fig. 3).

Nitrogen was transported from nodules to plant shoots mainly in the form of ureides (Fig. 4). These compounds represented from 65 (Rio Tibagi/127 K-17) to 90% (Negro Argel/C05) of the total nitrogen transported in plants grown without mineral nitrogen. Applying a low level of nitrogen [2.5 mg N(plant)$^{-1}$ day^{-1}] decreased the amino acid fraction slightly and the ureide fraction by an average of 25%, with a corresponding increase in the transport of nitrate in the sap.

The most efficient symbioses transported more ureides, as shown by the high correlation (r= 0.983**)

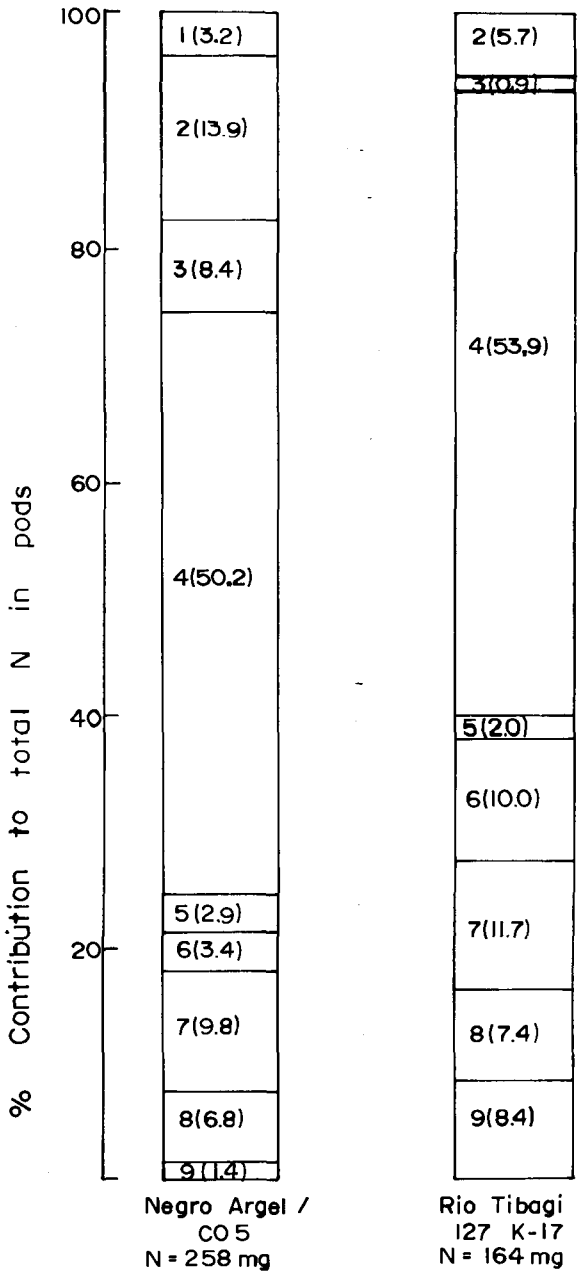

Fig. 3. Origin of N in the pods of the symbiotic systems Negro Argel/C05 and Rio Tibagi/127 K-17. Plants received 2.5 mg N(plant)$^{-1}$ day^{-1} and were harvested at 70 days after emergence (DAE). Numbers represent means of 4 replicates. Numbers in parentheses represent the percentual contribution of each component. F (flowering, 35 DAE); MPF (mid-podfill, 50 DAE); M (maturity, 70 DAE). The numbers from 1 to 4 represent Ndfa: 1) N fixed until F and remobilized from F to MPF; 2) N transported directly to pods from F to MPF; 3) N fixed until MPF and remobilized after MPF; 4) N transported directly to pods after MPF. Numbers from 5 to 9 represent Ndff; 5) N absorbed until F and remobilized from F to MPF; 6) N absorbed until F and remobilized after MPF; 7) N absorbed from F to MPF and remobilized after MPF; 8) N transported directly to pods from F to MPF; 9) N transported directly to pods after MPF.

between the percentage of ureide-N in the xylem sap at flowering and the total nitrogen in seeds derived from fixation. At the high level of nitrogen in the nutrient solution, nitrate became the dominant form of nitrogenous solutes in sap (70% of total nitrogen), whereas ureides represented not more than 5%. Both inoculation with strain C05 and addition of mineral N increased the transport of total nitrogen in the xylem sap (Fig. 4), but this was related to an increase in the rate of exudation and not to an increase in the nitrogen concentration.

Discussion

Results from initial screenings amongst commercial cultivars have shown that cv. Negro Argel nodulates well, whereas cv. Rio Tibagi responds to added nitrogen and nodulates poorly (Duque et al. 1985). In the present experiment, although cv. Rio Tibagi benefited from inoculation with the more efficient strain C05, it still responded significantly to mineral nitrogen. Thus, the seed yields of plants receiving the equivalent of 160 kg N ha^{-1} out-yielded those only inoculated and receiving the equivalent of 0 or 40 kg N ha^{-1}. On the other hand, seed yields of cv. Negro Argel, when associated with strain C05, reached maximum yields at the low nitrogen level.

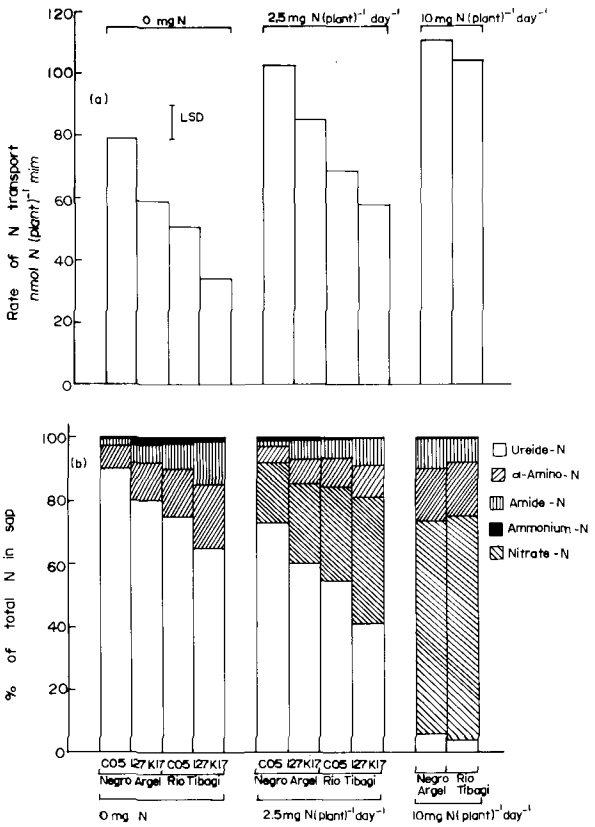

Fig. 4. Rate of N transport in the xylem sap (a) and N sap composition (b) of cvs Negro Argel and Rio Tibagi inoculated and/or receiving mineral N. The xylem sap was collected at flowering (35 days after emergence). Means of 4 replicates, LSD for $P = 0.01$ (Tukey).

Synergistic effects of mineral nitrogen on nitrogen fixation have been reported by Ruschel and Ruschel (1975), Ruschel et al. (1979) and Westermann et al. (1981). However, the present results show that, although mineral nitrogen stimulated plant growth, it decreased nitrogen fixation as estimated by nitrogenase activity or the ^{15}N dilution method.

Applied nitrogen promoted vegetative growth more than seed yield of cv. Negro Argel, but the same effect was less clear with Rio Tibagi, which requires nitrogen fertilizer in order to produce high seed yield. Similar effects of mineral nitrogen on nitrogen partitioning favouring the accumulation of nitrogen in the vegetative parts of the plant have already been observed in beans (Westermann et al. 1985), cowpeas (Summerfield et al. 1977, Neves et al. 1982) and soybeans (Weber 1966, Neves et al. 1985). The results obtained in the present study with cv. Negro Argel support earlier reports (Weber 1966) that nitrogen derived from fixation can be more efficiently used for seed nutrition than mineral nitrogen. The more mobile nature of the nitrogen derived from fixation was confirmed by the lower percentage of Ndfa in senesced leaves. Furthermore, it was observed that not only plant cultivars but also bacterial strains affected the remobilization of nitrogen from vegetative structures, as has been shown by Zeiher et al. (1982), Morris and Weaver (1983) and Neves et al. (1985).

Contrary to the general belief that rates of nitrogen fixation decline soon after flowering (Lawn and Brun 1974, Bethlenfalvay et al. 1978, Patterson and La Rue 1983), the data on nitrogenase activity and total nitrogen derived from fixation, show that nitrogen fixation continues at high rates during the seed filling stage, as was also observed in soybeans (Thibodeau and Jaworski 1975). The Ndfa comprised 58 to 62% of the total nitrogen fixed during the plant growth cycle. In this way, as has already been observed in beans (Hungria and Neves 1986a) and soybeans (Peat et al. 1981), the presence of pods can stimulate nitrogen fixation, due to the high rates of nitrogen metabolism in these organs ("sink" effect).

Although large differences in nitrogenase activity were detected between the symbiotic systems, the pattern of nitrate reduction was the same for all host-*Rhizobium* combinations, showing that an efficient symbiotic system can benefit from both a low level of mineral nitrogen and nitrogen fixation. More than 90% of the mineral nitrogen was reduced in shoots and the contribution of stems to the total nitrate reductase activity was high (33 to 45%, data not shown), which emphasises the importance of considering the activity of this organ (Andrews et al. 1984). In contrast to the observations of Schlesier (1977), the contribution of pods to total nitrate reductase activity was also high (50% of the

total activity in shoots from 45 to 55 DAE, data not shown). The uptake of mineral nitrogen was mainly between the flowering and mid-podfilling stages, when the plants assimilated from 47 to 58% of the total mineral nitrogen absorbed during the growth cycle.

The ureides were the most abundant forms of nitrogenous compounds transported in the xylem sap of nodulated plants, as has been shown by Cookson et al. (1980) and Thomas et al. (1984). The high correlation between the percentage of ureide-N in the xylem sap at flowering and the Ndfa in seeds confirm results previously reported for soybeans (Neves et al. 1985) and beans (Hungria and Neves 1986b). On the other hand, the addition of mineral nitrogen decreased the ureide-N and increased the nitrate-N fractions in the sap. These observations might explain the differences in the utilization of fixed and mineral nitrogen for pod-filling, as it has been proposed by Ishizuka et al. (1970) and Ohyama et al. (1981) that the amino acids are preferentially used for vegetative growth and ureides are mainly used for reproductive growth.

Although the best cultivar/strain combination used in the present study responded to low nitrogen doses, it did not show further seed yield increases with high mineral doses. Therefore, emphasis should be placed on the selection of plant genotypes and *Rhizobium* strains adequate to produce high seed yields with nitrogen from fixation, as the use of nitrogen fertilizers is not economically viable for the farming systems of developing countries. The differences found in the mobilization and partitioning of nitrogen in bean plants once more emphasises the importance of observing the symbiotic system as a whole and not only the individual partners.

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