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Effect of different levels of mineral nitrogen on nodulation and N_2 fixation of two cultivars of common bean (*Phaseolus vulgaris* L.)

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Abstract

An experiment was conducted under greenhouse conditions to evaluate the effect of mineral nitrogen on N_2 fixation of two cultivars of *Phaseolus vulgaris* L., Puebla 152 and Negro Argel. Nitrogen application was 0, 2.5, 12.5 and 25 mg N kg^{-1} of a vermiculite-sand-mixture at planting time. Shoot and root growth were elevated by nitrogen application at all growth stages. During vegetative growth (V 5) nodule dry weight and nitrogenase activity (acetylene reducing activity) per plant were reduced by nitrogen supply in both cultivars, but less in Negro Argel than in Puebla 152. At later stages nodulation in nitrogen-treated Puebla 152 did not differ from that in non-treated plants, whereas increased nodule number was found in Negro Argel at high nitrogen levels. The influence of mineral N on the total amount of nitrogen fixed in the two bean cultivars was only slightly different.

Introduction

Common bean (*Phaseolus vulgaris* L.) is considered to have poor symbiotic N_2 fixation potential compared to other legumes (Hardy et al., 1968; Piha and Munns, 1987). Field studies have shown that N fertilization increased yield substantially, indicating that the symbiotic nitrogen fixation is unable to provide enough nitrogen for maximum yield (Buttery et al., 1987; Huntingdon et al., 1986). Because of the weak performance of the symbiotic system in *Phaseolus vulgaris*, N fertilization is a common practice in most areas especially where growth conditions are sub-optimal.

In Brazil, fertilizer recommendations include applications of up to 20 kg N ha^{-1} at planting time alone, or together with a supplemental side-dressing of 40 kg N ha^{-1} during vegetative growth, depending on soil type, soil nutrient status and cropping history (Santana, 1985). It is thought that low N application may stimulate N_2 fixation by improving early plant growth until N_2 fixation provides adequate N for plant growth and development. On the other hand, fertilizer rates exceeding those exerting a "starter-N" effect, generally have reduced nodulation and N_2-fixation. In common bean the threshold for the depressing effect of mineral N seems to be low (Graham, 1981). Therefore, plants supporting a symbiotic system with a high tolerance against mineral N would be desirable. In the present study two cultivars of *Phaseolus vulgaris*, Puebla 152 and Negro Argel, were compared for their symbiotic performance and N_2-fixation in response to different amounts of mineral N applied at the time of sowing.

Material and methods

The experiment was conducted in the greenhouse of EMBRAPA-CNPAF in Goiânia, Brazil using the common bean cultivars, Puebla 152 and Negro Argel, both considered to have high N_2 fixation potential.

The nitrogen treatment were 0, 2.5, 12.5 and 25.0 mg N kg^{-1} of substrate applied as KNO_3 at planting. At each N level replicated pots with non-nodulating soybean isoline 'Harosoy' were included in the experiment. Two plants were grown in each 7 kg pot containing a N-free 2:1 vermiculite:sand mixture. 100 mL per pot of a N-free modified Summerfield's nutrient solution (Summerfield et al., 1977) was applied every two days and distilled water was supplied to maintain optimum moisture of the substrate. All the pots received 0.4 mL suspension with 10^8 cell of the *Rhizobium leguminosarum* biovar *phaseoli* strain CIAT 899 at planting.

Plant samples were taken at the V_5, R_1, R_7 growth stages to evaluate nodule number, nodule dry weight, shoot dry weight, shoot N content and nitrogenase activity by the acetylene reduction method (Hardy et al., 1968). Non-nodulating soybeans were harvested at R_7 stage to estimate the amount of N_2 fixed by common bean using the N difference method (Boddey, 1987). The experimental design was a randomized complete block design. Analysis of variance was done by MSTAT statistical programme and the Duncan's Multiple Range test was used for mean separation.

Results and discussion

Shoot dry weight and, even more, root dry weight, increased at all sampling dates when nitrogen was applied, with Negro Argel showing a greater response than Puebla 152 to mineral N (Table 1). Since N was applied at seeding time, available N decreased gradually. This is reflected by a strong effect on plant growth at early stages, and a smaller influence at later stages. Plant dry weight of Puebla was higher than that of Negro Argel, but percentage of N in the shoot of Puebla was less. Therefore, no cultivar differences in nitrogen accumulation per plant could be found (Table 1).

Nodule number of Puebla was mostly higher than that of Negro Argel, except at levels of high nitrogen application, but individual nodule dry weight was generally less (Table 2). The mineral N decreased nodule size, nodule dry weight and nitrogenase activity (acetylene reducing activity)

Table 1. Shoot and root dry weight, nitrogen content of shoot and N_2 fixed (N difference method) of two cultivars of *Phaseolus vulgaris* L., Puebla 152 (P.) and Negro Argel (N.A.), grown with two levels of mineral nitrogen at different growth stages (V5, R1, R7). Values in parenthesis: Percent N_2 fixed of total N

mg N kg^{-1} substrate	V_5		R_1		R_7	
	P	N.A.	P	N.A.	P	N.A.
Shoot dry weight (g $plant^{-1}$)						
0	1.38 c	0.92 d	3.83 b	2.93 c	11.61 bc	10.31 c
25	1.99 a	1.66 b	5.32 a	4.77 a	13.55 a	13.28 ab
Root dry weight (g $plant^{-1}$)						
0	0.20 b	0.12 c	0.39 b	0.25 b	0.49 bc	0.38 c
25	0.35 a	0.34 a	0.78 a	0.68 a	0.95 a	0.62 b
Nitrogen content in shoot (mg $plant^{-1}$)						
0	33.7 b	29.5 a	99.4 b	95.0 b	221.1 b	205.9 b
25	51.3 a	44.9 a	117.9 ab	140.3 a	228.7 ab	281.3 a
Nitrogen fixed (mg $plant^{-1}$)						
0					212 a	197 a
					(96)	(95)
25					189 a	242 a
					(82)	(86)

Means with different letters indicate significant differences ($p < 0.05$) among cultivars and N-treatments at one sampling date.

Table 2. Nodule number, nodule size and specific acetylene reducing activity of two cultivars of *Phaseolus vulgaris* L., Puebla 152 (P.) and Negro Argel (N.A.), grown with four levels of mineral nitrogen at different growth stages (V_5, R_1, R_7)

mg N kg^{-1} substrate	V_5		R_1		R_7	
	P	N.A.	P	N.A.	P	N.A.
Nodule number per plant						
0	198 a	110 c	405 ab	282 b	559 ab	347 b
2.5	186 ab	115 c	398 ab	367 ab	550 b	486 ab
12.5	155 abc	126 bc	335 b	393 ab	543 ab	434 ab
25.0	103 c	108 c	339 a	472 a	575 ab	610 a
Nodule D.W. (mg D.W. $nodule^{-1}$)						
0	0.52 b	0.86 a	0.61 c	0.88 a	0.65 ab	1.00 ab
2.5	0.55 b	0.85 a	0.61 c	0.72 abc	0.67 ab	0.64 ab
12.5	0.36 bc	0.73 a	0.72 abc	0.82 ab	0.70 ab	0.90 ab
25.0	0.27 c	0.51 b	0.58 c	0.67 bc	0.64 ab	0.55 b
Specific acetylene reducing activity (μmol C_2H_4 $g^{-1}h^{-1}$ nodule D.W.)						
0	18.0 ab	16.7 b	7.36 a	8.29 a	1.14 bc	1.18 bc
2.5	20.4 ab	18.0 ab	8.77 a	8.39 a	1.07 c	0.93 c
12.5	21.0 a	19.5 ab	9.49 a	8.25 a	1.25 bc	1.39 abc
25.0	17.2 ab	20.2 ab	8.86 a	8.42 a	1.60 ab	1.82 a

Means with different letters indicate significant differences ($p \leq 0.05$) among cultivars and N-treatments at one sampling date.

per plant during the growth phase until V5 (Fig. 1, 2). The effect was stronger in Puebla. A positive response was found in Negro Argel for nodule dry weight per plant at higher N levels at R_1 stage (Fig. 1), resulting from an increased nodule number at later stages (Table 2). Mineral nitrogen also caused elevated specific acetylene reducing activity in nodules at R_7 growth stage (Table 2). These results confirm positive effects of starter-N application on nodulation as described previously (Park and Buttery, 1989; Srivastava and Ormond, 1986).

A non-nodulating soybean was included as a reference plant in this study to differentiate between N taken up from the substrate and N_2 fixed. Nitrogen fixation contributed strongly to

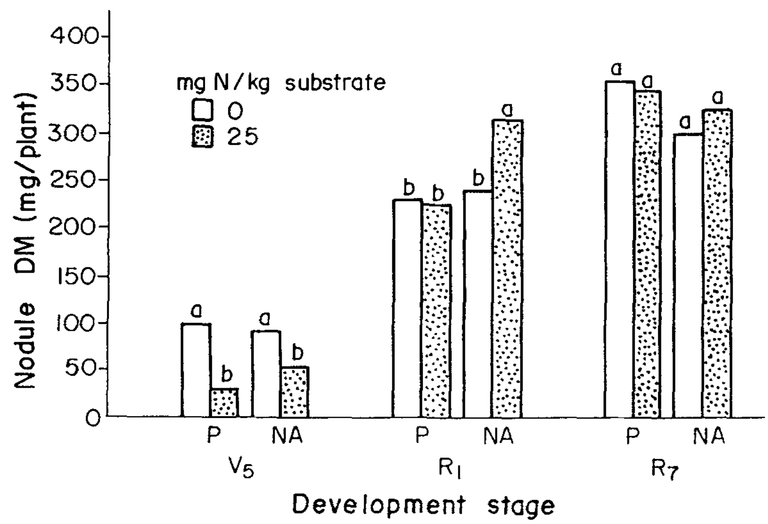

Fig. 1. Nodule dry weight of two cultivars of *Phaseolus vulgaris*, Puebla 152 (P) and Negro Argel (NA) grown with two levels of mineral nitrogen at different growth stages (V_5, R_1, R_7). Means with different letters indicate significant differences ($p \leq 0.05$) among cultivars and N-treatments at one sampling date.

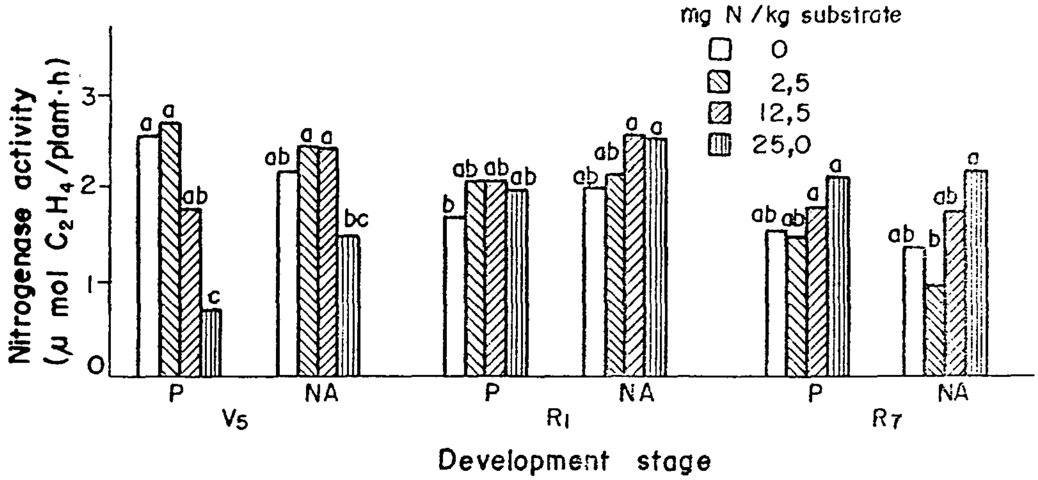

Fig. 2. Acetylene reducing activity of two cultivars of *Phaseolus vulgaris*, Puebla 152 (P) and Negro Argel (NA) grown with four levels of mineral nitrogen at different growth stages (V_5, R_1, R_7). Means with different letters indicate significant differences ($p \leq 0.05$) among cultivars and N-treatments at one sampling date.

the nitrogen accumulation of both bean cultivars at all nitrogen levels (Table 1). Although at R_1 Negro Argel had more nodule dry weight and nitrogenase activity on a per plant basis than Puebla, there was no significant difference between the two cultivars in the amount of N from fixation at R_7. This might result from a stronger increase of nodule dry weight per plant in Puebla than in Negro Argel after R_1 growth stage (Fig. 1). It is suggested, that the greater part of fixation takes place after flowering as previously reported (Zapata et al., 1987a,b). In spite of a decrease in percentage of nitrogen from atmosphere with increasing application of mineral nitrogen, the amount of N_2 fixed was not influenced significantly.

Studies with common bean and soybean have indicated that the sensitivity of N_2 fixation to combined N also depends on rhizobial strain (Awonaike et al., 1980; Senaratne et al., 1987). In pea (*Pisum sativum* L.) isolates of *Rhizobium* varied in effectiveness in the presence of combined N, but differences in symbiotic response were small and it was concluded that efforts to improve nitrogen fixation in the presence of combined nitrogen should be directed towards host cultivars rather than *Rhizobium* strains (Nelson, 1987). In common bean currently much effort is being made to identify and characterize genotypes with superior nodulation and N_2 fixation (Pereira et al., 1989; Rennie and Kemp, 1983, Ruschel et al., 1982; Wolyn et al., 1989). The results of the present study provide evidence for the variability of bean cultivars grown with different amounts of mineral N. It seems worthwhile to continue identifying lines that can use both sources of N effectively, to obtain parents for breeding programmes to improve N_2 fixation in beans.

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