SEÇÃO III - BIOLOGIA DO SOLO

SEEDS ENRICHED WITH PHOSPHORUS AND MOLYBDENUM IMPROVE THE CONTRIBUTION OF BIOLOGICAL NITROGEN FIXATION TO COMMON BEAN AS ESTIMATED BY ^{15}N ISOTOPE DILUTION[1]

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SUMMARY

Seeds with a high concentration of P or Mo can improve the growth and N accumulation of the common bean (*Phaseolus vulgaris* L.), but the effect of enriched seeds on biological N_2 fixation has not been established yet. This study aimed to evaluate the effect of seeds enriched with P and Mo on growth and biological N_2 fixation of the common bean by the ^{15}N isotope dilution technique. An experiment was carried out in pots in a 2 x 3 x 2 x 2 factorial design in randomized blocks with four replications, comprising two levels of soil applied P (0 and 80 mg kg^{-1}), three N sources (without N, inoculated with rhizobia, and mineral N), two seed P concentrations (low and high), and two seed Mo concentrations (low and high). Non-nodulating bean and sorghum were used as non-fixing crops. The substrate was 5.0 kg of a Red Latosol (Oxisol) previously enriched with ^{15}N and mixed with 5.0 kg of sand. Plants were harvested 41 days after emergence. Seeds with high P concentration increased the growth and N in shoots, particularly in inoculated plants at lower applied P levels. Inoculated plants raised from high P seeds showed improved nodulation at both soil P levels. Higher soil P levels increased the percentage of N derived from the atmosphere (%Ndfa) in bean leaves. Inoculation with the selected strains increased the %Ndfa. High seed P increased the %Ndfa in inoculated plants at lower soil P levels. High seed Mo increased the %Ndfa at lower soil P levels in plants that did not receive inoculation or mineral N. It is concluded that high seed P concentration increases the growth, N accumulation

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and the contribution of the biological N_2 fixation in the common bean, particularly in inoculated plants grown at lower soil P availability.

Index terms: molybdenum, nodulation, *Phaseolus vulgaris*.

RESUMO: *SEMENTES ENRIQUECIDAS COM FÓSFORO E MOLIBDÊNIO AUMENTAM A CONTRIBUIÇÃO DA FIXAÇÃO BIOLÓGICA DE NITROGÊNIO EM FEIJOEIRO ESTIMADA PELA DILUIÇÃO ISOTÓPICA DE ^{15}N*

*Sementes com altos teores de P ou Mo podem estimular o crescimento e a acumulação de N no feijoeiro (*Phaseolus vulgaris *L.), mas o efeito de sementes enriquecidas na fixação biológica de N_2 ainda não foi estabelecido. O presente estudo teve por objetivo avaliar o efeito de sementes enriquecidas com P e Mo no crescimento e na fixação biológica de N_2 do feijoeiro por meio da técnica de diluição isotópica de ^{15}N. Foi conduzido um experimento em vasos em esquema* fatorial *2 x 3 x 2 x 2 em blocos casualizados com quatro repetições, combinando duas doses aplicadas de P (0 e 80 mg kg^{-1}), três fontes de N (sem N, inoculado com rizóbio e N mineral), dois teores de P na semente (baixo e alto) e dois teores de Mo na semente (baixo e alto). O feijoeiro não nodulante e sorgo foram utilizados como controles não fixadores. O substrato constituiu-se de 5,0 kg de* Latossolo Vermelho *previamente* enriquecido com ^{15}N *misturado com 5,0 kg de areia. As plantas foram colhidas aos 41 dias após emergência. Sementes com alto teor de P aumentaram a acumulação de biomassa e de N da parte aérea, principalmente nas plantas inoculadas na menor dose de P no solo, e a massa de nódulos nas plantas inoculadas nas duas doses de P no solo. A maior dose de P no solo aumentou a porcentagem de N derivado da atmosfera (%Ndfa) nas folhas de feijoeiro. A inoculação com as estirpes recomendadas aumentou a %Ndfa. O alto P na semente aumentou a %Ndfa, nas plantas inoculadas sob baixo P no solo. O alto Mo na semente aumentou a %Ndfa sob baixo P no solo nas plantas que não receberam inoculação ou N mineral. Conclui-se que sementes enriquecidas com P aumentam o crescimento, a acumulação de N e a contribuição da fixação biológica de N_2 em feijoeiro, particularmente em plantas inoculadas crescidas sob menor disponibilidade de P no solo.*

Termos de indexação: molibdênio, nodulação, Phaseolus vulgaris.

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) has been considered of low biological N_2 fixation capacity as compared to other grain legumes, which is attributed to the poor soil conditions where the crop is usually grown, the susceptibility of the species to nutritional and environmental stresses, the short vegetative period available for N_2 fixation, the sensitivity of the symbiosis to soil nitrate, and the competition from indigenous but often ineffective soil rhizobia (Piha & Munns, 1987; Leidi & Rodríguez-Navarro, 2000; Graham et al., 2003; Hungria et al., 2003). Nevertheless, ranges of biologically fixed N_2 in field-grown bean lines were reported as from 25 to 65 kg ha^{-1} (Ruschel et al., 1982) or 40 to 125 kg ha^{-1} (Rennie & Kemp, 1983) in shoots at physiological maturity, or from 21 to 44 kg ha^{-1} in grains (Pereira et al., 1989). Additionally, field experiments in seven countries showed that the N_2 fixation contributed with 35 % of the N accumulated by bean crops, with the highest figures at 70 % (Hardarson et al., 1993). Yet, using the ^{15}N natural abundance method, Kimura et al. (2004) estimated contributions of N_2 fixation ranging from 24 to 50 % in field-grown bean at different growth stages. Such evidence justifies efforts for improving the contribution of symbiotic N to the crop.

Low nutrient availability and soil acidity, typical of weathered soils, constitute serious restrictions to N_2 fixation in the common bean grown in the tropics (Graham et al., 2003). In acid soils, Mo is rapidly fixed to Fe and Al oxides and bound to organic matter, and the recovery from P fertilizers by crops is usually very low because most P becomes unavailable due to adsorption, precipitation or conversion to organic forms. The N_2 fixation under P deficiency is often reduced, which is usually explained by an effect of low P supply on the growth of the host plant, on the growth and functioning of the nodule, or on the growth of both plant and nodule (Almeida et al., 2000). Low P supply could induce changes in the relative growth of nodules and shoots rather than changes in leaf photosynthesis or N_2-fixing activity per unit nodule mass (Hogh-Jensen et al., 2002). In addition to the P demand of the host plant, nodules require relatively higher amounts of P and energy than other plant tissues (Vadez et al., 1999; Olivera et al., 2004; Mortimer et al., 2008). Nodules of bean plants act as strong sinks of P and Mo to guarantee adequate rates of N_2 fixation (Brodrick & Giller, 1991; Almeida et al., 2000; Christiansen & Graham, 2002).

Seed constitutes a significant source of nutrients for seedlings and developing plants. Seed Mo content is sometimes enough for adequate growth during the whole growth season, and seed P content, although constituting a small proportion of total plant demand, may also be influential for plant establishment (Tyler & Zohlen, 1998). Moreover, nutrient-enriched seeds of leguminous plants can stimulate symbiotic N_2 fixation. Common bean plants raised from seeds with high P concentration showed increased growth, nodulation and N accumulation, particularly at low soil P levels (Teixeira et al., 1999; Araújo et al., 2002). Since the limited P supply retards the development of nodulation in the common bean (Araújo & Teixeira, 2000), P-enriched seeds can provide P during initial stages of nodule formation and growth (Thomson et al., 1991). Bean plants originating from seeds with high Mo concentration had higher accumulation of biomass and N in shoots and higher root nitrogenase activity (Brodrick & Giller, 1991; Kubota et al., 2008) and also produced more grains in soil with low N content particularly when plants did not receive additional foliar Mo (Vieira et al., 2005). In spite of such encouraging results, studies evaluating the simultaneous seed enrichment with P and Mo are not available.

Among the techniques suitable to quantify plant-associated N_2 fixation, the ^{15}N isotope dilution has been used widely for providing estimates of the proportion of N derived from N_2 fixation integrated over time (Unkovich et al., 2008). This technique involves the application of ^{15}N labeled fertilizer to the soil, and subsequent harvest of the N_2-fixing and non-fixing control crops, which should both obtain N from the soil with the same ^{15}N enrichment (Unkovich et al., 2008). Hence, this work aimed to evaluate the effect of seeds enriched with P and Mo on the growth and biological N_2 fixation of common bean plants by the ^{15}N isotope dilution technique.

MATERIALS AND METHODS

Bean seeds of the Carioca cultivar enriched with P and Mo were obtained in a field experiment carried out at Santa Mônica Farm of Embrapa Gado de Leite (Valença - RJ), where different combinations of foliar fertilization with P and Mo were tested. At 52 and 71 days after seed emergence, 5 kg ha^{-1} of P as $NH_4H_2PO_4$ and 120 g ha^{-1} of Mo as $(NH_4)_6Mo_7O_{24}.4H_2O$ were foliar sprayed. Plots also received foliar-applied urea to homogenize N levels. Seed samples suffered nitro-perchloric acid digestion, and in the extracts P concentration was measured colorimetrically by molybdenum-ascorbic acid, and Mo concentration by plasma emission spectrometry (ICP-EAS, Perkin-Elmer) (Table 1).

Table 1. Concentration of P and Mo, and 100-seed mass, of common bean seeds of the Carioca cultivar tested in the experiment

Seeds	P concentration	Mo concentration	100 seed mass
	mg g^{-1}	µg g^{-1}	g
Low P low Mo	2.5	nd	21.4
Low P high Mo	2.7	12.2	20.6
High P low Mo	3.6	n.d	21.6
High P high Mo	3.9	5.8	21.4

nd: values lower than 0.5 µg g^{-1} Mo, not detected by plasma spectrometry.

The experiment was conducted at Embrapa Agrobiologia (Seropédica - RJ), from October to December 2007. A 2 x 3 x 2 x 2 factorial scheme in randomized block design with four replications was used, comprising two levels of P applied to the soil (0 and 80 mg kg^{-1} of P), three N sources (control without N, seeds inoculated with rhizobia, and mineral N), two seed P concentrations (low and high) and two seed Mo concentrations (low and high). Non-nodulating bean genotype NORH-54 and sorghum cultivar BR-501 were used as non-fixing crops, which were also grown at two soil applied P levels (0 and 80 mg kg^{-1}) with four replications.

The substrate was a 4 mm sieved Ap horizon of a clayey Red Latosol (Latossolo Vermelho) from Piracicaba - SP. The soil had received 10 kg ha^{-1} of N as ammonium sulphate with 10 atom % ^{15}N in excess in a field experiment conducted several years before, and was stored in sacks during the last few years. The soil presented 0.14 atom % ^{15}N in excess at the beginning of the experiment. Hence, interferences on the method due to spatial and temporal changes in ^{15}N enrichment of the soil mineral N during plant growth was rather reduced (Unkovich et al., 2008). Soil chemical analysis, performed as described by Embrapa (1997), showed: water pH 5.1, 0.0 mmol$_c$ dm^{-3} of Al^{3+}, 42 mmol$_c$ dm^{-3} of Ca^{2+}, 11 mmol$_c$ dm^{-3} of Mg^{2+}, 28 mg dm^{-3} of available P (Mehlich-1), 121 mg dm^{-3} of available K. The soil was mixed with washed sand (1:1 in weight basis) and placed into 10 kg/pots. The following nutrients were applied diluted in water (in mg kg^{-1} of substrate): 10 Mg as $MgSO_4.7H_2O$, 2 Cu as $CuSO_4.5H_2O$, 1 Zn as $ZnSO_4.7H_2O$, 0.05 B as H_3BO_3, and 80 P as KH_2PO_4 at high soil P level. Pots of low P level also received 75 mg kg^{-1} of K as KCl to uniform the K supply. In the treatments with mineral N, pots also received 60 mg kg^{-1} of N as urea. The substrate of each pot was then homogenized. At sowing, the substrate presented 12 and 49 mg dm^{-3} of available P (Mehlich-1), respectively, at the applied P levels of 0 and 80 mg kg^{-1}.

Seeds were sown six days after soil fertilization. In inoculated treatments, each seed received 1 mL of liquid inoculant containing the strains CIAT899 (or BR322) and PR-F81 (or BR520) of *Rhizobium tropici* from the collection of Embrapa Agrobiologia. Four bean plants were grown per pot after trimming. Pots were placed in open air, on tiles distributed on a greensward, and irrigation was performed daily. Twenty days after emergence, 300 mg N was applied per pot as urea in mineral N treatments. Abscised leaves were not collected during the experiment. Plants were harvested 41 days after emergence by the stage of pod setting. At harvest, shoots were cut at ground level, and leaves and stems were separately processed. Roots and nodules were recovered by washing the soil through a 2 mm sieve. Leaves, stems, roots, and nodules were oven dried, weighed and finely ground using a roll-mill.

Total N concentration was measured in each plant portion by the semi-micro Kjeldahl procedure. Accumulation of N was obtained by the product of N concentration and dry mass. The ^{15}N isotope composition was measured in one replication per treatment (except those that received mineral N) in leaves, stems and roots, using a continuous-flow isotope-ratio mass spectrometer (Finnigan DeltaPlus, Finnigan MAT, Bremen, Germany) at Embrapa Agrobiologia. Leaves and stems showed similar ^{15}N enrichment at both soil P levels (Table 2), and leaves corresponded to 54 % of the total N accumulated by the plants at harvest, averaged across all treatments. Therefore, the N isotopic composition was further measured only in leaves of bean plants under inoculation or without N, and in shoots of the non-nodulating bean and sorghum, of all replications. The percentage of N derived from the atmosphere (%Ndfa) was estimated by the formula (Unkovich et al., 2008):

$$\%Ndfa = [1 - (\text{atom}\%\ ^{15}N \text{ excess of bean/atom }\%\ ^{15}N \text{ excess of non-fixing})] \times 100$$

The analysis of variance was performed as a four-factor design, evaluating the effects of soil P level, N source, seed P concentration, seed Mo concentration, and their interactions. The Tukey test at a 0.05 level was used to compare treatment means.

Table 2. Enrichment of ^{15}N (in atom % ^{15}N in excess) of different plant tissues; means of eight replications for bean tissues, and four replications for non-fixing crops, at two P levels applied to the soil (0 and 80 mg kg^{-1})

Plant tissue	Soil P level (mg kg^{-1})	
	0	80
Bean tissue		
Leaves	0.0449 Ab	0.0227 Bb
Stems	0.0408 Ab	0.0316 Bb
Roots	0.0735 Aa	0.0478 Ba
Shoot of non-fixing crops		
Non-nodulating bean	0.1177 Ab	0.1320 Ab
Sorghum	0.1503 Aa	0.1570 Aa

Lowercase letters compare plant parts in columns (within bean tissue or non-fixing crops), and capital letters compare soil P levels in lines; means followed by the same letter did not differ by t test at 0.05 level.

RESULTS AND DISCUSSION

The analysis of variance identified significant effects of the main factors of soil P level, N source and seed P concentration for shoot dry mass, nodule dry mass and N accumulation in shoots, whereas the effect of seed Mo concentration was significant for shoot N accumulation. Triple interaction between N source, seed P concentration and seed Mo concentration was significant for shoot mass, and the quadruple interaction was significant for nodule mass and for N accumulation in roots. Therefore, the means of each treatment are presented in figures to guide the interpretation of such complex effects, and the least significant difference calculated by the Tukey test at 0.05 level is presented to compare seed treatments within each soil P level and N source.

Plant growth

The highest P level applied to the soil strongly increased the shoot growth of bean plants, particularly in the control and inoculated plants (Figure 1a). Averaged across seed treatments, the highest soil P level increased shoot mass by 100 and 89 % in control and inoculated plants, respectively, whereas the highest P level increased shoot mass only by 16 % in plants with mineral N. It confirms that plants relying on symbiotic N had a larger demand for P to obtain an optimal growth than plants supplied with mineral N (Cassman et al., 1981; Israel, 1987). Plants under mineral N had greater shoot mass than the control and inoculated plants at both soil P levels, but the improved growth associated to mineral N was more intense at low soil P levels (Figure 1a). Control and inoculated plants yielded similar shoot mass.

At the lowest soil P level, inoculated plants raised from seeds with high-P high-Mo or with high-P low-Mo had greater shoot mass than plants from seeds with low-P low-Mo (Figure 1a). At the lowest soil P level, plants receiving mineral N originating from seeds with low-P high-Mo had lower shoot mass than those from seeds with high-P high-Mo. At the highest soil P level, control plants originating from seeds with high-P low-Mo had higher shoot mass than those from low-P low-Mo (Figure 1a). Therefore, the effect of seed P concentration on plant growth was more intense for inoculated plants at a lower soil P level. Teixeira et al. (1999) and Araújo et al. (2002) also observed that bean plants originating from P-enriched seeds showed improved shoot mass, but this effect was reduced as P level applied to the soil was increased. The effects of Mo-enriched seeds on shoot growth were of small magnitude (Figure 1a). These results contrast with those of Kubota et al. (2008), which reported improved shoot growth of bean plants raised from seeds with high Mo concentration.

Nodule mass was strongly increased by a higher soil P supply (Figure 1b). Averaged across seed and N treatments, nodule mass increased by 101 % and shoot mass by 50 % when P was applied to the soil. It confirms that traits related to biological N_2 fixation are more responsive to soil P supply than plant growth (Almeida et al., 2000; Araújo & Teixeira, 2000). Even control plants showed a strong nodulation (Figure 1b), which reinforces the argument that the common bean is a promiscuous host regarding native soil rhizobia (Hungria et al., 2003). The addition of mineral N strongly reduced nodulation at both soil P levels (Figure 1b) since the *Phaseolus-Rhizobium* symbiosis is quite sensitive to soil nitrate (Westermann et al., 1981; Leidi & Rodríguez-Navarro, 2000). However, at the highest soil P level nodulation was less affected by the mineral N applied. Indeed, at the highest soil P level plants under mineral N had fewer but larger nodules, with an individual nodule mass of 2.79 mg/nodule, as compared to 2.32 and 2.07 mg/nodule in the control and inoculated plants, respectively, averaged across seed treatments. Leidi & Rodríguez-Navarro (2000) observed that the increased P supplies improved nodulation of bean plants at low N concentrations in nutrient solutions, whereas the effect of P in high N concentrations was negligible.

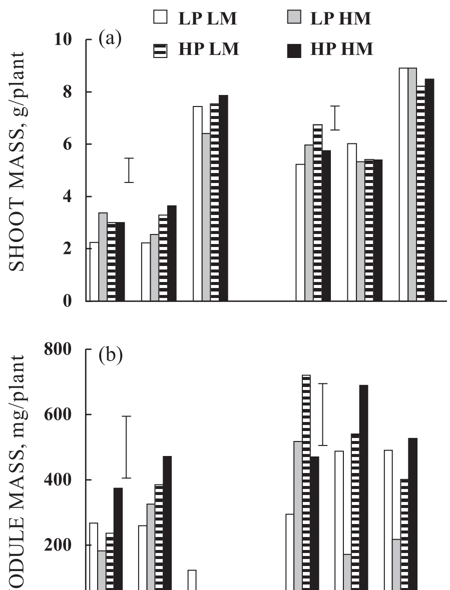

Figure 1. Shoot (a) and nodule (b) mass of common bean plants grown at two P levels applied to the soil (0 and 80 mg kg^{-1}) and three N sources (CT control without N, IN inoculated with rhizobia, MN mineral N), originating from seeds with different concentrations of P and Mo (LP low P, HP high P, LM low Mo, HM high Mo); vertical bars represent the least significant difference by the Tukey test at 0.05 level, and compare seed treatments within each N source and soil P level.

Inoculated plants raised from high-P high-Mo seeds had higher nodule mass than plants raised from low-P low-Mo seeds at both soil P levels (Figure 1b). For control plants, high-P high-Mo seeds yielded more nodule mass than low-P high-Mo seeds at low soil P levels, whereas high-P low-Mo seeds yielded more nodule mass than the other seed treatments at high soil P levels (Figure 1b). Teixeira et al. (1999) verified that seeds with high P concentration enhanced nodulation of bean plants at every soil P level, but this effect was more intense at low soil P. High seed P concentration stimulated nodulation of lupin (*Lupinus angustifolius* L.) at every level of external P supply, but particularly under P deficiency (Thomson et al., 1991).

Seeds with low-P high-Mo concentration reduced nodule mass of plants that received inoculation or mineral N at the highest soil P level (Figure 1b). For control plants, low-P high-Mo seeds reduced nodule mass as compared to high-P high-Mo seeds at low soil P level, and as compared to high-P low-Mo seeds at high soil P level (Figure 1b). Kubota et al. (2008) also observed that bean plants originating from seeds with high Mo concentration showed reduced nodulation, although nitrogenase activity of root systems was not affected by seed Mo concentration. Since Mo supply is usually associated with increased nitrate reductase activity in bean plants (Pessoa et al., 2001), it is hypothesized that the additional Mo provided by enriched seeds would stimulate the assimilation of the N taken up from the soil, hence impairing nodule growth by a possible feedback mechanism induced by high concentration of reduced N compounds in roots (Leidi & Rodríguez-Navarro, 2000; Hogh-Jensen et al., 2002).

Accumulation of N

Plants that received mineral N showed similar N accumulation in shoots at both P levels, contrasting with control or inoculated plants where shoot N accumulation was higher when P was applied to the soil (Figure 2a). It demonstrates the higher P demand for N acquisition of plants depending on N_2 fixation (Israel, 1987).

Seeds with high P concentration increased N accumulation in shoots of inoculated plants at the lowest soil P level (Figure 2a). High-P low-Mo seeds increased shoot N accumulation of control plants at the highest soil P level. Teixeira et al. (1999) also verified that bean plants originating from seeds with high P concentration had larger amounts of N accumulated in shoots. Seeds with high-P high-Mo increased N accumulation in the root systems of inoculated plants, as compared to low-P low-Mo seeds at low soil P, and as compared to low-P high-Mo seeds at high soil P level (Figure 2b). The highest N accumulation in the root system occurred in plants raised from high-P high-Mo seeds that received mineral N at the highest soil P level (Figure 2b), indicating a synergistic effect of enriched seeds and soil N and P supplies upon N acquisition.

Seeds with low-P high-Mo concentration reduced shoot N accumulation in plants that received mineral N at the lowest soil P level, and also reduced shoot and root N accumulation in inoculated plants at the highest soil P level (Figure 2a,b). Despite the reduced N accumulation in shoots of inoculated plants raised

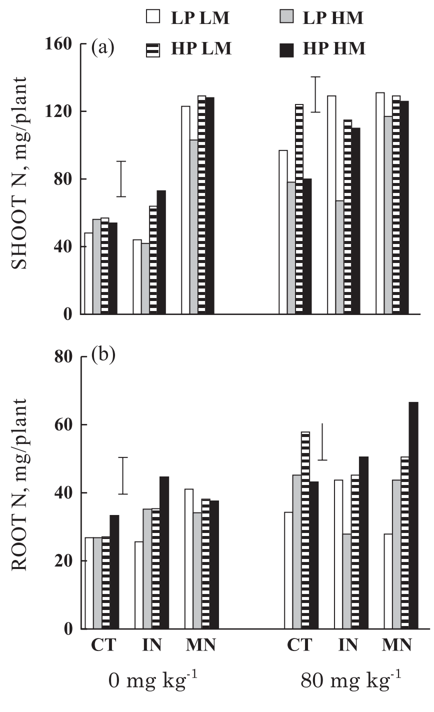

Figure 2. Accumulation of N in shoots (a) and roots (b, roots plus nodules) of common bean plants grown at two P levels applied to the soil (0 and 80 mg kg^{-1}) and three N sources (CT control without N, IN inoculated with rhizobia, MN mineral N), originating from seeds with different concentrations of P and Mo (LP low P, HP high P, LM low Mo, HM high Mo); vertical bars represent the least significant difference by the Tukey test at 0.05 level, and compare seed treatments within each N source and soil P level.

from low-P high-Mo seeds at the highest soil P level (Figure 2a), shoot mass was not reduced (Figure 1a), denoting an effect of seed Mo in diluting N in shoots. It could indicate a preferential effect of seed Mo on nitrate reductase activity, increasing N assimilation thus reducing plant demand for further N acquisition. Such hypothesis deserves further experimental work.

^{15}N isotope dilution

There was no significant effect of soil P levels on ^{15}N enrichment of non-fixing crops (Table 2). However, even using a homogenized soil supposedly stable in ^{15}N enrichment with time, non-nodulating bean and sorghum differed in terms of ^{15}N enrichment (Table 2). This reinforces the need to use different control crops to check the stability of soil ^{15}N labeling in order to provide more accurate estimates of N_2 fixation (Viera-Vargas et al., 1995). As non-nodulating beans best resemble the growth pattern of N_2-fixing common beans, they were used as non-fixing crop in calculating the percentage of N derived from the atmosphere (%Ndfa) for the nodulated legume. The ^{15}N enrichment was similar in stems and leaves of bean plants, but roots showed higher ^{15}N enrichment (Table 2). It denotes that the N absorbed from the soil was accumulated rather by roots than shoots, whereas in shoots the N taken from the soil was diluted by symbiotically fixed N_2.

Estimates of the %Ndfa ranged from 54 to 79 % (Table 3). Such figures are similar to those reported by Rondon et al. (2007), ranging from 50 to 72 %, but they are slightly higher than those reported by Viera-Vargas et al. (1995), varying between 49 and 61 %, for bean plants grown in pots with soil labeled with a single application of ^{15}N. Using the ^{15}N natural abundance, Mortimer et al. (2008) verified that %Ndfa in mycorrhizal beans grown in a nutrient solution

Table 3. Contribution of biological N_2 fixation (in % of N derived from the atmosphere) in leaves of common bean plants at 41 days after emergence, grown at two levels of P applied to the soil (0 and 80 mg kg^{-1}) and two N sources (control without N and inoculated with rhizobia); means of two P and two Mo concentrations in sowing seeds

N source	Percentage of N derived from the atmosphere in leaves		
	0	80	Mean
	mg kg^{-1}		
Control	53.5 B	75.7 A	64.6 b
Inoculated	59.0 B	78.7 A	68.8 a
Mean	56.2 B	77.2 A	

Lowercase letters compare N sources in columns, and capital letters compare P levels in lines; means followed by the same letter, or without letters, did not differ by Tukey test at 0.05 level.

ranged from 50 to 55 % at 31 days after emergence. Hardarson et al. (1993) observed the highest values of %Ndfa in field-grown bean lines (70 %) when environmental conditions were favorable.

Inoculated plants had higher %Ndfa in leaves than control plants that did not receive inoculation or mineral N (Table 3). Therefore, in spite of the intense nodulation of control plants (Figure 1b), the inoculation of selected rhizobia strains contributed to improve N_2 fixation in common bean. Hungria et al. (2003) also verified that inoculation with selected strains increased N_2 fixation of field-grown common beans, expressed by higher N content in shoots and higher percentage of N as ureides. Higher soil P level increased the %Ndfa in bean leaves (Table 3). The ^{15}N isotope dilution technique used in field experiments also indicated that %Ndfa rises as soil P level increases in the common bean (Ssali & Keya, 1983) and lentil (Badarneh, 1995) crops. However, the %Ndfa in field-grown faba beans (*Vicia faba*) was not affected by P fertilization (Amanuel et al., 2000).

Seeds enriched with P increased the %Ndfa but this effect was significant only for inoculated plants at the lowest soil P level (Table 4). It indicates that seeds enriched with P, associated to inoculation of selected strains, can improve the contribution of N_2 fixation in the common bean, particularly in soils with low P availability. To our knowledge, it is the first report of enhanced contribution of N_2 fixation yielded by P-enriched seeds of legume plants. High-Mo seeds increased the %Ndfa but only in plants that did not receive N or inoculation at the lowest soil P level (Table 4). It is supposed that the higher %Ndfa in inoculated over control plants (Table 3) has reduced the additional effect of seed Mo on N_2 fixation of inoculated plants (Table 4). Yet in control plants, with lower diazotrophic efficacy of indigenous rhizobia strains, seed Mo would have stimulated N_2 fixation in a larger extent (Table 4).

These results demonstrate the beneficial effects of P-enriched seeds on improving growth and biological N_2 fixation of common bean plants. The effects of Mo-enriched seeds were less consistent than those reported by Vieira et al. (2005) and Kubota et al. (2008). Hence, the advantage of the simultaneous seed enrichment with P and Mo remains inconclusive. However, only one measurement at the stage of pod setting was performed in the present work, and other studies should be carried out in field conditions to ensure the effects of enriched seeds over the entire growth season.

CONCLUSIONS

1. Common bean plants accumulate more N and produce larger biomass of shoots and nodules when raised from seeds with high P concentration.

2. Seeds enriched with Mo increase the contribution of biological N_2 fixation in the common bean but only in plants that did not receive inoculation or mineral N grown at lower soil P levels.

3. Seeds enriched with P increase the accumulation of N in shoots and the contribution of biological N_2 fixation in the common bean, particularly for inoculated plants grown at lower soil P levels.

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Table 4. Contribution of biological N_2 fixation (in % of N derived from the atmosphere) in leaves of common bean plants at 41 days after emergence, grown at two levels of P applied to the soil (0 and 80 mg kg^{-1}) and two N sources (control without N and inoculated with rhizobia), originating from seeds with low or high P and Mo concentrations

	Percentage of N derived from the atmosphere in leaves					
	Control			Inoculated		
	Low Mo	High Mo	Mean	Low Mo	High Mo	Mean
			0 mg kg^{-1} of P			
Low P	43.5	62.4	52.9	45.2 b	51.5 b	48.4 b
High P	50.2	57.8	54.0	68.4 a	71.0 a	69.7 a
Mean	46.9 B	60.1 A		56.8	61.2	
			80 mg kg^{-1} of P			
Low P	77.1	77.9	77.5	79.7	75.8	77.7
High P	76.6	71.4	74.0	81.0	78.1	79.6
Mean	76.8	74.7		80.4	76.9	

Lowercase letters compare seed P concentrations in columns, and capital letters compare seed Mo concentrations in lines; means followed by the same letter, or without letters, did not differ by the Tukey test at 0.05 level.

inoculantes para culturas alimentares e agro-industriais" and of Embrapa Agrobiologia through the project "Aumento dos teores de fósforo e molibdênio em sementes de feijoeiro via adubação foliar visando beneficiar a fixação biológica de nitrogênio". We also thank Dr. Daniel Vidal Pérez, of Embrapa Solos (Rio de Janeiro - RJ) for seed analysis.

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