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Nitrogen and molybdenum fertilization and inoculation of common bean with *Rhizobium* spp. in two oxisols

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ABSTRACT. The aim of this study was to evaluate the effects of foliar application of molybdenum and small dose of nitrogen at planting on common bean production when using seed inoculation with rhizobia. Two field experiments were conducted, applying a randomized block design and a factorial arrangement (3x2x2) + 1, with three replicates. Treatments consisted of inoculation with *Rhizobium* spp. (strain CIAT 899^T, strain UFLA 02–100, and uninoculated), foliar application of molybdenum (0 and 80 g ha^{-1}), and fertilization with N-urea at planting (0 and 20 kg ha^{-1}), with an additional N treatment of 40 applied at planting and 40 kg ha^{-1} applied as topdressing. The foliar application of molybdenum did not favor the nodulation, but its effect on yield was dependent on the chemical soil characteristics, providing highest yield in soil at Patos de Minas with pH more acid and with higher phosphorus and organic matter concentrations. Small dose of nitrogen applied at planting did not reduce nodulation of bean inoculated with rhizobia, but it can reduce the seeds emergence, thus negatively affecting yield. The native rhizobia promoted nodulation, the plant growth, shoot N accumulation and yield similar to the treatments that received inoculations of rhizobia strains.

Keywords: *Phaseolus vulgaris* L., biological nitrogen fixation, nutrients.

Adubações nitrogenada e molíbdica e inoculação com *Rhizobium* spp. no feijoeiro-comum em dois latossolos

RESUMO. Objetivou-se avaliar os efeitos da aplicação foliar de molibdênio e do emprego de pequena dose de nitrogênio no plantio sobre a produção do feijoeiro quando se utiliza a inoculação das sementes com rizóbio. Foram conduzidos dois experimentos em campo, em delineamento de blocos ao acaso, esquema fatorial (3x2x2) + 1, com três repetições. Os tratamentos foram inoculação (estirpe CIAT 899^T, estirpe UFLA 02–100 e ausência de inoculação), aplicação foliar de molibdênio (0 e 80 g ha^{-1}) e de nitrogênio na semeadura (0 e 20 kg ha^{-1}), mais tratamento adicional com N (40 na semeadura e 40 kg ha^{-1} na cobertura). A aplicação foliar de molibdênio não favoreceu a nodulação, mas seu efeito sobre o rendimento de grãos foi dependente das características químicas do solo, fornecendo maior produtividade em solo de Patos de Minas, com pH mais ácido e maiores teores de fósforo e matéria orgânica. Pequena dose de nitrogênio no plantio não reduziu a nodulação do feijoeiro inoculado com rizóbio, mas pode reduzir a emergência das sementes, com reflexo negativo na produtividade. Os rizóbios nativos proporcionaram nodulação, crescimento vegetal, acúmulo de nitrogênio na parte aérea e rendimento equivalentes aos tratamentos inoculados com as estirpes de rizóbio.

Palavras-chave: *Phaseolus vulgaris* L., fixação biológica de nitrogênio, nutrientes.

Introduction

Brazil is the largest producer and consumer of common bean in the world. Besides, this legume species is an important protein source in the Brazilian population's diet.

Nitrogen (N) is the nutrient consumed by and exported in the bean plant in the greatest quantities. Given the substantial losses of N by volatilization, by leaching, and by the rapid decomposition of organic matter, and the high economic and ecological costs of N fertilizers, biological nitrogen fixation (BNF) is a rational solution for meeting the N requirements of bean crops.

Applications of small amounts of N to the soil have been reported to result in the growth of larger nodules and increase BNF (Brito, Muraoka, & Silva, 2011). However, applications of high doses of N, especially at planting, negatively affect nodulation and BNF (Soares et al., 2006; Souza, Soratto, & Pagani, 2011), and may also affect the germination and the emergence of common bean (Kikuti, Andrade, Carvalho, & Morais, 2005; Alves Júnior, Andrade, Carvalho, Vieira, & Morais, 2009). For the state of Minas Gerais, under field conditions, it has not been yet established a safe N dose in the planting furrow that does not reduce nodulation and BNF, and not have the salt effect of nitrogen fertilizer.

In addition to N, molybdenum (Mo) also has a large effect on BNF because the main functions of Mo in the plant are related to N metabolism, specifically the nitrogenase enzyme system present in the bacteroids in nodules and the nitrate reductase enzyme system that is essential for plant N assimilation (Dechen & Nachtigall, 2007). Thus, the application of molybdenum can improve the nitrogen efficiency applied to the crop and then reducing the doses of this macronutrient.

Common bean is usually known for a low BNF efficiency because of limitations by some biotic and abiotic factors. In recent years, an increasing number of studies have demonstrated that bean crops can benefit from BNF under field conditions, provided that the management of the factors related to seed inoculation are improved (Ferreira et al., 2009; Rufini et al., 2011). On the other hand, the application of a small dose of N at planting, together with inoculation with efficient *Rhizobium* spp. strains and foliar fertilization with Mo can be important tools for increasing bean yields and profitability for farmers (Vieira, Ronzelli Júnior, Daros, Koehler, & Prevedello, 2000; Valadão et al., 2009; Albuquerque, Pegoraro, Vieira, Amorim, & Kondo, 2012). However, the low number of studies does not allow reliable conclusions, mainly related to Mo application.

The aim of this study was to evaluate the effects of foliar application of Mo and small dose of N at planting on common bean production when using seed inoculation with rhizobia in Red Latosol (Oxisol).

Material and methods

Two field experiments were conducted during the 2011/2012 spring-summer growing season, in areas owned by the Minas Gerais Agricultural Research Corporation (Empresa de Pesquisa Agropecuária de Minas Gerais - Epamig), one in Patos de Minas in the Alto Paranaíba (Sertãozinho Experimental Farm) and one in Pitangui (Pitangui Experimental Farm), in the midwestern region of the state of Minas Gerais, Brazil. According to the Köppen classification system, the climate of the two regions belongs to the CWA type, which is described as a highland tropical climate with a hot and wet summer and a cold and dry winter (Vianello & Alves, 1991).

Both experiments were established using a conventional cultivation system, with one plowing and two harrowings, in areas with no previous records of inoculation of bean plants or of other legumes species. The preceding crops were corn and beans in Patos de Minas and pasture in Pitangui. The soil in Patos de Minas was classified according Brazilian classification (Empresa Brasileira de Pesquisa Agropecuária [Embrapa], 2013) as a Eutroferric Red Latosol with a loamy texture and that in Pitangui as a Dystroferric Red Latosol (Table 1). Both are oxisols according USDA classification.

Table 1. Results of the chemical analyses of soil samples (0-20 cm) collected before planting at Patos de Minas and Pitangui, state of Minas Gerais State.

Location	Characteristics[1]											
	pH (H_2O)	Available P mg dm^{-3}	K	Ca	Mg	Al	SB	t	T	m	V	OM dag kg^{-1}
			cmol$_c$ dm^{-3}								%	
Patos de Minas	5.0	73.8	0.13	0.9	0.3	0.3	1.3	1.3	8.3	18.4	15.9	3.1
Pitangui	5.8	9.4	0.54	5.3	1.5	0.1	7.3	7.4	10.9	1.3	67.1	2.2

[1]SB: Sum of exchangeable bases; t: Cation exchange capacity; T: Cation exchange capacity at pH = 7; m: Exchangeable aluminum; V: Base saturation; OM: Organic matter.

A randomized block experimental design was implemented, with three replicates. Treatments were arranged as a factorial (3x2x2) + 1: types of inoculation (*Rhizobium tropici* strain CIAT 899^T, *Rhizobium etli* strain UFLA 02–100, and uninoculated), two doses of Mo (0 and 80 g ha^{-1}), doses of N applied at planting (0 and 20 kg ha^{-1}) plus an additional N treatment of 40 at planting and 40 kg N ha^{-1} as topdressing between stages V3 and V4 of the common bean crop cycle (Fernandez, Gepts, & López, 1985).

Strain CIAT 899^T (Graham et al., 1994) is one of the strains approved by the Ministry of Agriculture, Livestock and Supply for use as a commercial inoculant for bean seeds. Strain UFLA 02-100 was isolated from the state of Rondônia and has been shown to be highly efficient at inoculation in Leonard jars (Pereira et al., 2000) and in soils from the state of Minas Gerais (Soares et al., 2006; Ferreira et al., 2009).

Molybdenum (as sodium molybdate p.a.) was applied foliarly between stages V3 and V4, at 20 days after emergence (DAE). The Mo solution was applied using a manual backpack sprayer at a dose of 80 g Mo ha^{-1} and a spray volume of 400 L ha^{-1}.

The nitrogen fertilization at sowing was performed manually in the planting furrow, and mixing the urea with the soil before receiving the seed. In Pitangui the fertilizer was incorporated by immediate irrigation and in Patos de Minas there was no incorporation. In additional N treatment, the N topdressing (urea), also applied manually, was distributed in a continuous stream along the side of the plant rows, between stages V3 and V4.

The furrows of all of the plots were fertilized with 70 kg P_2O_5 ha^{-1} (triple superphosphate source) in Patos de Minas and 110 kg P_2O_5 ha^{-1} (simple superphosphate source) in Pitangui. At both locations, 40 kg K_2O ha^{-1}, using potassium chloride as the source, was also applied, as Recommendations for use of amendments and fertilizers in Minas Gerais State, (Ribeiro, Guimarães, & Alvarez, 1999). Liming was not performed.

Inoculants were prepared with peat that had been sterilized in an autoclave that was mixtured at a ratio of 3:2 (w:w) with log phase cultures of each strain in semi-solid 79 medium (Fred & Waksman, 1928). The procedures were similar to those described by Soares et al. (2006). The resulting material was applied at 100 g per 10 kg of seeds. The quality of the inoculant was monitored by counting the number of colony-forming units (CFU) and comparing it to the legal minimum number of viable cells, approximately 10^9 *Rhizobium* cells per gram of inoculant, at planting. Immediately after inoculation, seeds from the 'BRSMG Madrepérola' cultivar (Carioca grain) were manually sown (11/23/2011 in Patos de Minas and 01/25/2012 in Pitangui) at a density of 17 seeds m^{-1}.

In relation to the cultivation practices adopted, pest and disease control was not required. Weeds were controlled using manual weeding whenever necessary. Only the experiment at Pitangui was irrigated, using self-propelled sprinklers that, during crop establishment, did not provide a uniform application of water.

Each plot consisted of six 4-m-long rows spaced 0.5 m apart (12 m^2). Rows 1 and 6 were designated as borders; rows 2 and 3 were used for sampling during flowering; and rows 4 and 5 were harvested at maturation.

At 49 DAE, during full flowering (stage R6), 10 plants were collected from each plot to measure the number (NN, unit 10 plants^{-1}) and dry weight of the nodules (NDW, g 10 plants^{-1}) as well as the shoot dry weight (SDW, g 10 plants^{-1}) and the shoot N accumulation (SNA, g 10 plants^{-1}).

At maturation (stage R9), which occurred at 96 and 91 DAE in Patos de Minas and in Pitangui, respectively, the final stand (FS, thousand plants ha^{-1}) and the yield (kg ha^{-1}) were measured. The final stand was obtained by counting the number of plants from the usable area. The yield corresponded to the production measured for all of the plants in rows 4 and 5, and the grain weight was corrected to account for the 130 g moisture kg^{-1}. The shoot N accumulation was calculated by multiplying the shoot dry weight by the shoot total N content (STN) and then dividing by 100. The STN was measured using the semi-micro-Kjeldahl method (total N), as performed by Sarruge and Haag (1979).

All of the data were subjected to individual and combined analyses of variance using the Sisvar version 4.0 software (Ferreira, 2011), after being previously subjected to normality (Shapiro-Wilks test) and variance homocedasticity (Bartlett test) tests, using the R software (R Development Core Team, 2011). The viability of the combined analysis was measured by comparing the magnitude of the residual average square of the individual analysis as described in Banzatto and Kronka (2006). Comparisons between the levels for location, Mo dose, and N dose were performed using the F-test because these factors have only two levels. In cases with significant interactions or contrasts, the main effects of the location and the experimental factors were obviously not discussed. Comparisons between additional treatment and levels of each factor were performed using Dunnett's test (Banzatto & Kronka, 2006). To meet the assumptions of the analysis of variance, the data for the number and dry weight of the nodules were $(x+1)^{0.5}$ transformed.

Results and discussion

The mean values of number and dry weight of nodules did not differ between inoculation treatments (uninoculated and UFLA 02-100 and CIAT 899^T strains; Figure 1A and B). This finding indicates that the native bacteria were able to promote nodulation in both soils, resulting in equal mean values for the variables mentioned above, which has also been observed in other studies (Kaneko et al., 2010; Souza et al., 2011).

The values of number and dry weight of nodules for Patos de Minas were greater than those for Pitangui, regardless of the Mo dose, applied foliarly (Figure 2A and B). This result may be at least partially attributed to the more favorable conditions for nodulation at the former, which had a pH more acid and higher phosphorus and organic matter concentrations than the values obtained in Pitangui (Table 1), likely favoring an increased availability of Mo in the soil.

There was a decrease in nodulation in Patos de Minas in response to the Mo application (Figure 2A and B), suggesting a phytotoxic effect of

Mo or of another ion in the Mo source used. At this location, the potential for a toxic level of Mo is supported by the high level of phosphorus (Table 1) because an increased level of P in a soil with a pH between 4.0 and 5.2 increases the absorption of Mo, thus indicating a synergistic effect of phosphorus (Jones Jr., Wolf, & Mills, 1991). A negative effect of Mo fertilization was not observed in Pitangui (Figure 2A and B) due to the higher pH of the soil (5.8), which increased the availability of the micronutrient and avoid the detrimental effects of its application (Ferreira, Andrade, & Araújo, 2004). Notably, regardless of the limiting factor for nodulation, at this location, these values were sufficiently low to mask any effect of Mo.

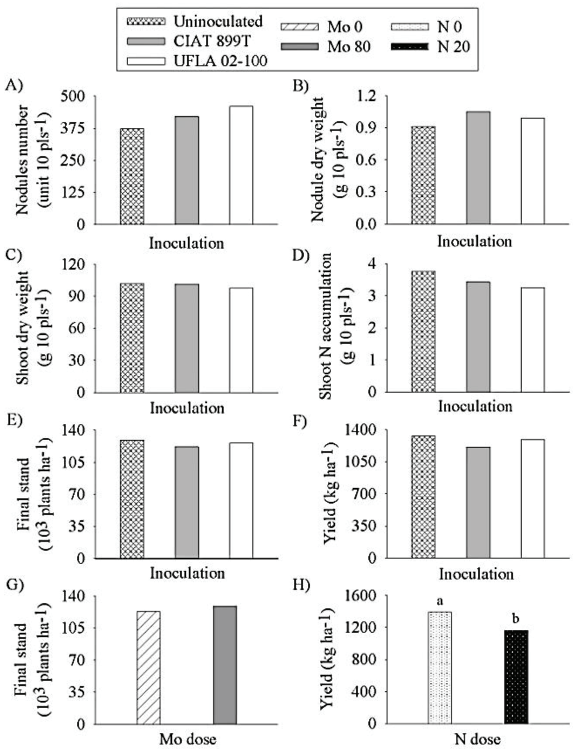

Figure 1. Mean number (A) and dry weight of nodules (B), dry weight (C) and N accumulation (D) in the shoots of the bean plant, final stand (E), and yield (F) according to inoculation, and final stand (G) and yield (H) according to Mo (g ha^{-1}) and N (kg ha^{-1}) doses, respectivily, for bean cultivar BRSMG Madrepérola. Different letters above the column indicate significant difference at a 5% probability level, by the F-test.

According to Moreira and Siqueira (2006), an high amount of mineral N in the soil reduces plant nodulation because of the lack of stimulus related to a nutritional deficiency. In this study, fertilization with 20 kg N ha^{-1} at planting did not affect the mean of number and dry weight of nodules because the low dose was insufficient to suppress nodulation. It can be seen also in the comparisons involving additional treatment, even the application of 80 kg N ha^{-1} (half at planting and half as topdressing) did not reduce nodulation; instead, it caused a fertilization effect of sufficient strength to increase the nodules dry weight (Figure 3).

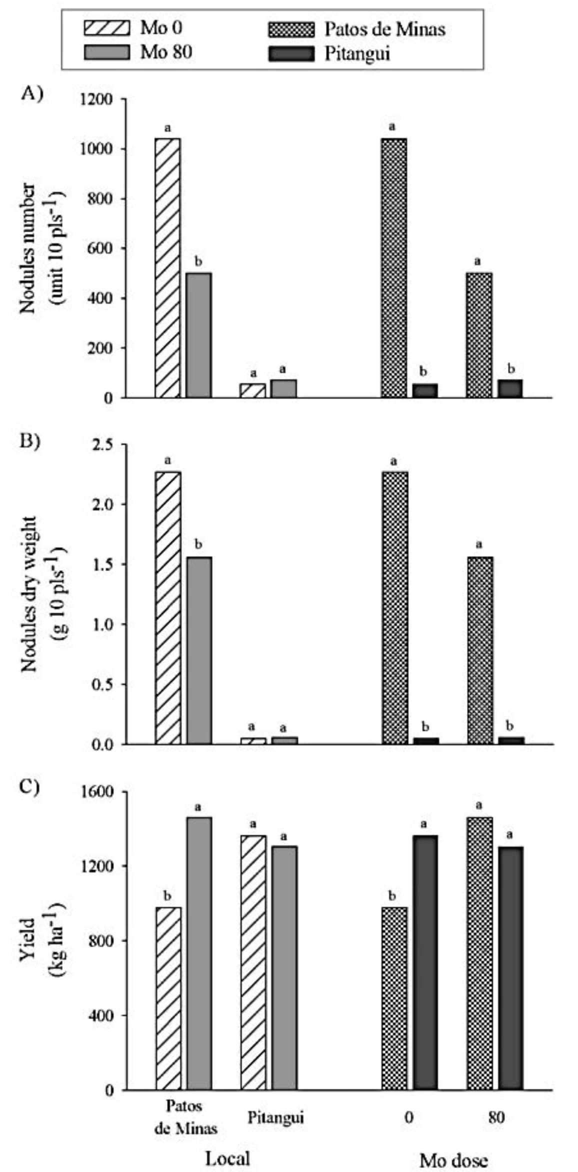

Figure 2. Mean values of the number (A) and dry weight (B) of nodules, and yield (C) according to location and molybdenum dose (g ha^{-1}). Different letters above the column, for each level in each factor, indicate significant difference at a 5% probability level, by the F-test.

Similarly, the mean values for shoot dry weight and shoot N accumulation did not differ between the inoculation treatments (Figure 1C and D). This finding indicates that, in these two soils, the activity of the native rhizobia populations can meet the plant is requirement for N, thus allowing the plants to grow similarly to the inoculated plants, regardless of the *Rhizobium* strain.

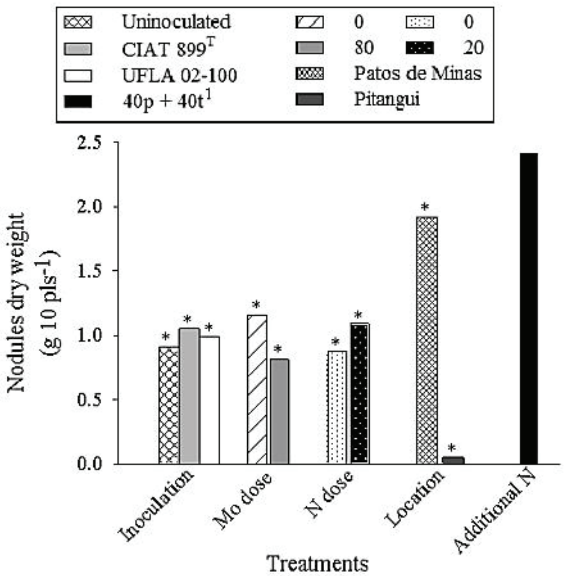

Figure 3. Mean values of the dry weight of nodules according to inoculation, molybdenum (g ha^{-1}) and nitrogen (kg ha^{-1}) dose, location and additional N treatment (kg ha^{-1}). *Indicate significant difference at a 5% probability level, by the Dunnett test, when compared with the additional N treatment. [1]Treatment fertilized with 80 kg ha^{-1} of N-urea (40 applied at planting + 40 kg N ha^{-1} applied as topdressing between stages V_3 and V_4 of the bean crop cycle).

An analysis of the L x N x Mo interaction shows that, in the majority of cases, N did not affect the shoot dry weight and shoot nitrogen accumulation (Figure 4). An atypical behavior was observed in Pitangui, where the application of 20 kg N ha^{-1} at planting, in the absence of Mo, resulted in a lower shoot dry weight than the value obtained in the absence of N fertilization, which did, however, affect shoot nitrogen accumulation (Figure 4B and D). This fact is likely attributed to the salt effect of urea, given that the irrigation was less uniform in the initial phase of the experiment in Pitangui. The salt effect of N fertilization at planting has been frequently reported in the literature to result in decreases in germination, emergence, and dry matter accumulation in common bean plants (Kikuti et al., 2005; Alves Júnior et al., 2009).

The effects of the foliar application of Mo on the shoot dry weight and shoot nitrogen accumulation varied according to the location and the N dose applied at planting. Bean plant growth was not affected by the application of Mo at Patos de Minas (pH = 5) (Figure 4A and C), but at Pitangui (pH = 5.8), the 80 g ha^{-1} dose of Mo increased these variables in the treatments with 20 kg N ha^{-1} (Figure 4B and D). This positive effect was not expected in Pitangui because of its higher pH, but it is possible that Mo fertilization partially alleviated the stress associated with the salt effect of the N fertilizer on the plants, leading to a partial restoration of N uptake.

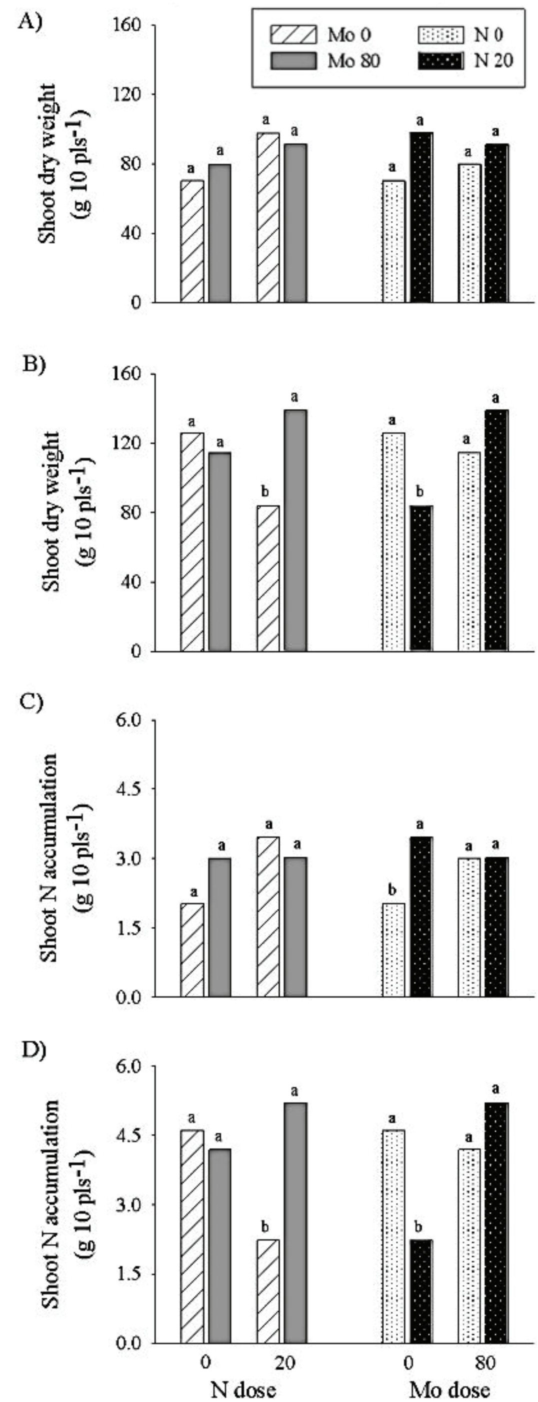

Figure 4. Dry weight (A and B) and N accumulation (C and D) in the shoots of bean plants, according to nitrogen (kg ha^{-1}) and molybdenum (g ha^{-1}) doses, in two locations (A and C – Patos de Minas; B and D – Pitangui). Different letters above the column, for each level in each factor and local, indicate significant difference at a 5% probability level, by the F-test.

For the shoot dry weight, in Patos de Minas, the application of 80 kg N ha^{-1} increased the mean values while in Pitangui not significant differences were observed (Figure 5A and B).

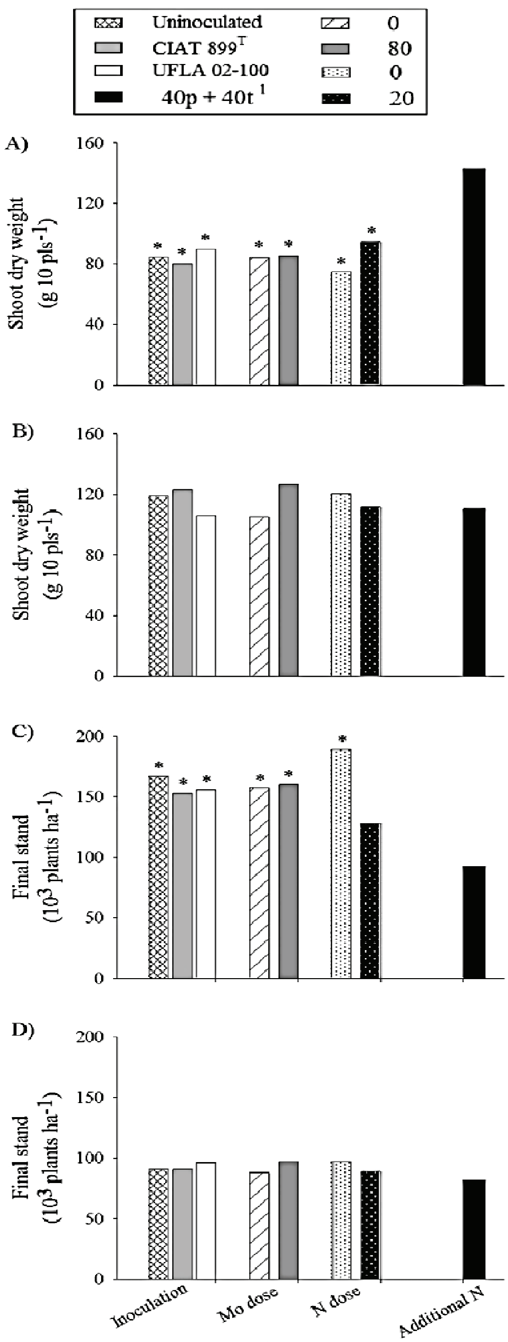

Figure 5. Mean values of the shoot dry weight (A and B) and final stand (C and D) according to inoculation, molybdenum (g ha^{-1}) and nitrogen (kg ha^{-1}) dose, and additional N treatment (kg ha^{-1}), in two locations (A and C – Patos de Minas; B and D – Pitangui). *Indicate significant difference at a 5% probability level, by the Dunnett test, when compared with the additional N treatment. [1]Treatment fertilized with 80 kg ha^{-1} of N-urea (40 applied at planting + 40 kg N ha^{-1} applied as topdressing between stages V3 and V4 of the bean crop cycle).

The mean final stand values did not exhibit any clear trend according to inoculation treatment or Mo dose and were similar (Figure 1E and G), as previously reported by Andrade, Alvarenga, Silva, Carvalho and Junqueira (2001). In relation to the N dose, the treatment effect was dependent on the location of the experiment. Regardless of N dose at planting, the number of plants was higher in Patos de Minas than in Pitangui. In this location the means were close to the 170,000 plants ha^{-1} recommended by Dourado Neto and Fancelli (2000) for type III bean plants. In Pitangui, the final stand was less than 100,000 plants ha^{-1}. Applying N at planting decreased the stand of the crop in Patos de Minas. In Pitangui, where the stand was already low, this effect was not significant, and other factors, such as the lack of uniformity of the initial water application, may have had an effect on this variable (Figure 6). The additional N treatment, with 40 kg N ha^{-1} (urea), at planting, resulted in a smaller final stand in Patos de Minas (Figure 5C and D). For this same characteristic and location, there was no significant difference between the doses of 20 and 80 kg N ha^{-1}.

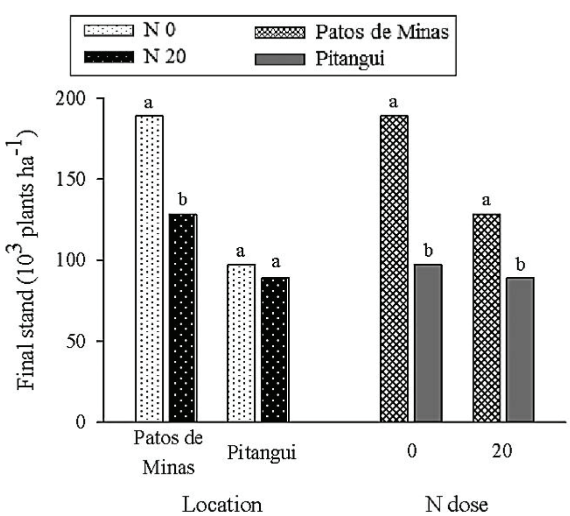

Figure 6. Final stand for bean plants, according to location and nitrogen dose (kg ha^{-1}). Different letters above the column, for each level in each factor, indicate significant difference at a 5% probability level, by the F-test.

As observed for the other traits, yield was not affected by seed inoculation (Figure 1F), although it was influenced by the main effects of N (Figure 1H) and Mo, with the latter dependent on the location, as evidenced by means for the L x Mo interaction (Figure 2C).

Both the introduced bacteria and the bacteria already established in the soil resulted in yields similar to the mean values recorded in the state of Minas Gerais during the 2011/12 rainy season, with a yield on the order of 1,200 kg ha^{-1} (Companhia Nacional de Abastecimento [Conab], 2012). Based on these results, both inoculations with an efficient strain and BNF by native rhizobia may contribute to reducing the amount of mineral N supply required for the crop, without affecting bean yield.

Treatments fertilized with 20 kg N ha^{-1} at planting produced lower grain yields (16% lower than the treatment without N; Figure 1H). This result can be explained by the reduction in the final plant population attributed to the effect of salt stress (Kikuti et al., 2005; Alves Júnior et al., 2009) and consequent decrease in grain production per area.

Conversely, the application of 80 g Mo ha^{-1} increased the grain yield by 33% in Patos de Minas, but this effect was not observed in Pitangui (Figure 2C). Positive effects of foliar applications of Mo on grain yield have also been reported by other authors. Calonego, Ramos Junior, Barbosa, Leite and Grassi Filho (2010) and Ferreira, Araújo, Cardoso, Fontes and Vieira (2003) observed increased grain production in treatments receiving approximately 80 g ha^{-1} of Mo applied foliarly. The isolation of the effect of foliar fertilization with Mo to Patos de Minas is explained by the lower pH of the soil in this location because the response to Mo is strongly dependent on pH-dependent soil reactions. At lower pH values, the nutrient is less available, favoring a response to its application (Calonego, Ramos Junior, Barbosa, Leite, & Grassi Filho, 2010; Rocha, Araújo, Carneiro, Cecon, & Lima, 2011). In soils with highest pH, such as in Pitangui, there is an increase in the availability of the Mo already present in the soil, thus preventing or reducing a response to fertilization.

Conclusion

The foliar application of molybdenum did not favor the nodulation, but its effect on yield was dependent on the chemical soil characteristics, providing highest yield in soil at Patos de Minas with pH more acid and higher phosphorus and organic matter concentrations.

Small dose of nitrogen applied at planting did not reduce nodulation of common bean inoculated with rhizobia, but it can reduce the seeds emergence, thus negatively affecting yield.

The native rhizobia populations provided nodulation to support plant growth, shoot N accumulation, and bean yield similar to the treatments that were inoculated with CIAT 899^T and UFLA 02-100 strains.

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