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Molybdenum and Cobalt Application in Bean (*Phaseolus vulgaris* L.) with Two Fertilization Systems under No-Tillage

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Abstract: Molybdenum (Mo) and Cobalt (Co) play an important role in nitrogen (N) metabolism of grain legumes. Their applications to alkaline soils have been scarcely studied. A field experiment was set up to evaluate the Mo and Co application in common bean cv. Canario Centenario under two fertilization systems: inorganic fertilization and inoculation with *Rhizobium etli*. In each system, Mo and Mo + Co were applied by seed impregnation at doses of 1.36 g/kg of seed for Mo and 0.26 g/kg of seed for Co; a control for each fertilization system and an absolute control were included. Yield components, nodules characteristics and N content by grains and foliage were evaluated. A completely randomized block design with seven treatments and five replications was used. Mo application increased the grain yield, grain size, nodulation and N accumulation in grains under both fertilization systems. Mo increased the total N uptake by the plant in 35.4% and 26%, for N fertilized and inoculated plants, respectively. Co application increased only nodules number and weight, and N content under inoculation. Mo application to beans cultivated in alkaline soils is recommended, but Co application is suitable only when beans are inoculated with rhizobia.

Key words: Mo, Co, N fertilization, *Rhizobium etli*, N content.

1. Introduction

Common beans are one of the most cultivated legumes in Peru, representing an average of 86 thousand ha yearly [1]. Despite its importance, its cultivation is mainly carried out by farmers with the limited access to economic and technological resources. Nitrogen (N) requirement of bean varies between 80 kg/ha and 100 kg/ha, although 150 kg/ha may be required for higher yields. If N fertilization comes from nitric source, the partaking of molybdenum (Mo) as cofactor is essential. And, if N fertilization is through *Rhizobium* sp., not only Mo but also cobalt (Co) is important [2].

Mo is a cofactor of several enzymes involved in the metabolism of N, e.g., nitrate reductase acting on the nitrate reduction to ammonia in the plant and nitrogenase involved in symbiotic N fixation [3]. So, increasing its activity could increase yield variables [3-6], biometrics parameters [4], N content [7] and nodules weight [2].

Co is part of cobalamin cofactor-dependent methionine synthase, ribonucleotide reductase and methylmalonyl CoA enzymes of *Rhizobium* sp., which influence in qualitative and quantitative characteristics of the root nodules [8, 9]. Then, the addition of those microelements to plants could increase crop [10, 11].

It is generally considered that Mo availability increases with soil pH [9], thus, alkaline soils with pH over 8, or limed soils are considered sufficient in Mo [8]. In contrast, Co availability is reduced by increasing soil pH. Then, under experimental field conditions with slightly alkaline pH, Co application would be beneficial to legumes inoculated with *Rhizobium*.

It was reported in Refs. [12-15] that the joint application of Mo and Co contributed to better results

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of the studied variables of legumes. However, in Refs. [13, 16, 17], best results were obtained when such micronutrients were applied separately. Most studies have been conducted in acidic soils, thus the benefits of Mo and Co application in alkaline soils remain unclear. It is proposed that the Mo and Co application by seed impregnation can increase yield and N content in beans under inorganic N fertilization and inoculation with *Rhizobium etli*. Thus, an experiment was set up at the field level, under a no-tillage system, with the aim of evaluating the effect of Mo and Co application on nodulation, biometric variables and grain yield of common bean.

2. Materials and Methods

The experiment was carried out at the experimental field of La Molina University (12°05′13″ S; 76°56′42″ W), La Molina, Province of Lima, Peru. The field soil has deep, moderate permeability, good drainage, loamy, fine granular structure and friable moist consistency. The parameters of the soil were measured as the following: electrical conductivity (EC) = 0.54 dS/m, pH = 8.1, soil organic matter = 1.18%, extractable P (Olsen) = 8.8 mg/kg, extractable K = 228 mg/kg, cation exchange capacity (CEC) = 18.38 cmol/kg.

La Molina has a hot desert climate with winter fogs. Average monthly temperature during the crop season (September to January) ranged from 14.9 °C to 23.9 °C, with the minimum and maximum temperatures of 12.5 °C and 27.7 °C during September and January, respectively. Cumulative rainfall and potential evapotranspiration were 6 mm and 502 mm, respectively. Thus, irrigation was applied during the entire crop season.

Corn (*Zea mays* L.) was cultivated in the field during the previous season (autumn), and common bean (*Phaseolus vulgaris* L.) cv. Canario Centenario (shrub type with bright yellow grains), was selected for the spring season. Beans were planted by direct seeding two weeks after corn harvest. Sowing was done at a row distance of 0.8 m and plant distance of 0.25 m, with three seeds in each hole, for a density of 150,000 plants/ha.

Two N fertilization systems were set up: inorganic fertilization and rhizobial inoculation. In each system, Mo and Mo + Co were applied by seed immersion at doses of 1.36 g/kg seed for Mo and 0.26 g/kg seed for Co. Ammonium molybdate [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 54% Mo] and cobalt sulfate ($CoSO_4\cdot 7H_2O$, 20% Co) were used as Mo and Co sources, respectively. Both salts were diluted in distilled water and bean seeds were immersed in the solutions 20 min before sowing. A control without micronutrient application for each fertilization system and an absolute control were also included, resulting in seven treatments.

In the inorganic fertilization treatments, N (80 kg/ha) was applied as ammonium nitrate. The dose was split in two fractions applied at 8 d and 50 d after the emergence of seeds of Canario Centenario bean. In the treatments with rhizobial inoculation, *Rhizobium etli* growing in a liquid medium containing 1×10^8 cells/mL was used. The inoculum was added with gum arabic and impregnated to seed at a dose of 0.625 mL/kg of seed. Both inorganic fertilization and rhizobial treatments were fertilized at planting with 60 kg/ha P_2O_5 and 80 kg/ha K_2O using triple superphosphate and potassium chloride, respectively. Soil moisture was maintained close to field capacity through weekly irrigation. Leafminer outbreaks were controlled with application of 0.1% abamectin, and 0.1% tebuconazole was applied before flowering for prevention of bean rust.

Treatments were applied to plots consisting in 5 m long sections of five rows (20 m^2). A completely randomized block design with seven treatments and five replications was used. Plant leaf area and nodule formation were evaluated at the beginning of flowering (58 d after sowing) in an evaluation area consisting in 4 m long sections of the three central rows in each plot. The number of nodules per plant and their weight were registered in 10 randomly selected roots. Grain yield and its components (number of pods per plant, number of grains per pod and 100-grain weight) were evaluated at harvest (132 d after sowing). Grain yield was calculated from the grain weight obtained from the plants in the evaluation area adjusted to 14% moisture. Grain components were evaluated from 10 plants randomly selected from the evaluation area. The shoot biomass was measured after harvest and harvest index was calculated.

Portions of shoots and grains were oven-dried at 70 °C to obtain the dry matter content, and then finely ground. N content was determined in both tissues by Kjeldahl digestion, and N partitioning in grains, shoots and the entire plant was calculated.

The values obtained from variables evaluation were further processed using ANOVA. The averages were compared by multiple comparison test of Tukey's honestly significant difference (HSD) with a significance level of 5%. The Statistical Analysis System Program (SAS) was used for statistical analysis [18].

3. Results and Discussion

Results about the biometric variables and yield of bean (plant leaf area, pods per plant, grains per pod, 100-grain weight and grain yield) are summarized in Table 1. Both inorganic N fertilization and rhizobial inoculation clearly increased leaf area, grain yield and its components, compared to unfertilized control, and inorganic N fertilization resulted in higher values compared to inoculated treatments. Inorganic N fertilization treatment with Mo addition showed the best result in all the above mentioned variables, among all treatments, probably due to the increase in nitrate reductase activity. It has been reported by Marschner [2] that applying Mo 5 μg/plant, the enzymatic activity increased at 27.3 times.

In the inoculated bean treatments, the bean inoculated with *Rhizobium etli* with Mo addition (RhMo) showed the best effect in the increasing of leaf area, harvest index and yield, highlighting the important role of Mo on nitrogenase and xanthine oxidase/dehydrogenase activity in the atmospheric N reduction and purine metabolism to ureide, respectively [2]. However, there was no complementarity between Mo and Co, as the Co was added to RhMo, in contrary, leaf area decreased slightly. But, there was an increasing of 3.75 nodules per plant and a slight increasing in seed yield (8.52 kg/ha); Co application just favored the two reservoirs. These effects could be explained by that Co forms an essential part of the cobalamin coenzyme, which is dependent on the three enzymes for nodulation: methylmalonyl-CoA mutase, ribonucleotide reductase

Table 1 Effect of Mo and Co addition under two systems of N fertilization on biometric traits and yield components of bean.

Treatments	Leaf area (dm^2)	No. of pods/plant	No. of grains/pods	The weight of 100 grains (g)	Total weight (kg/ha)	Harvest index (%)	Yield (kg/ha)
Control	8.22^{F}	8.80^{D}	3.20^{D}	40.19^{C}	$1,433.70^{F}$	47.00^{D}	679.74^{F}
Inorganic fertilization							
-	36.73^{C}	16.80^{C}	4.20^{ABC}	41.61^{BA}	$3,647.50^{C}$	52.00^{C}	$1,881.96^{C}$
Mo	45.23^{A}	32.40^{A}	5.00^{A}	42.35^{A}	$5,287.50^{A}$	51.00^{C}	$2,716.00^{A}$
Mo + Co	41.01^{B}	28.20^{B}	4.60^{AB}	42.22^{A}	$4,680.00^{B}$	51.00^{C}	$2,402.90^{B}$
Rhizobium etli (Rh)							
-	25.37^{E}	11.20^{D}	3.40^{CD}	41.31^{B}	$2,272.40^{E}$	52.00^{BC}	$1,191.92^{E}$
Mo	35.20^{C}	16.20^{C}	4.00^{BCD}	41.46^{BA}	$3,137.50^{D}$	55.00^{A}	$1,719.28^{D}$
Mo + Co	30.97^{D}	16.40^{C}	4.20^{ABC}	41.79^{BA}	$3,212.50^{D}$	54.00^{BA}	$1,727.80^{D}$

The values shown are average of five replications.

A-F Values within a column followed by the same letter are not significantly different for Tukey's HSD.

Table 2 Effect of Mo and Co addition under two systems of N fertilization on nodulation by *Rhizobium etli* and N content in bean plants.

Treatments	No. of nodules/plant	Nodules weight (mg/plant)	N in grains (g/m^2)	Stubble N (g/m^2)	Total N (g/m^2)
Control	25.70^C	2.10^D	1.90^F	0.63^C	2.61^G
Inorganic fertilization					
-	14.70^D	2.40^C	5.60^C	1.80^A	7.44^C
Mo	14.20^D	2.40^C	8.40^A	1.66^A	10.08^A
Mo + Co	16.50^{DC}	2.40^C	6.80^B	1.88^A	8.76^B
Rhizobium etli (Rh)					
-	40.20^B	2.30^{DC}	3.40^E	1.130^B	4.58^F
Mo	48.50^{BA}	2.70^B	4.80^D	0.960^B	5.77^E
Mo + Co	52.20^A	2.90^A	5.10^{DC}	1.150^B	6.26^D

The values shown are average of five repetitions.

$^{A\text{-}F}$ Values within a column followed by the same letter are not significantly different for Tukey's HSD.

and methionine synthase, involved in heme synthesis, DNA and protein, respectively [9]. Supplying Mo to the beans inoculated with *Rhizobium etli*, nodules number and weight increased in 88% and 37%, respectively, which could be explained by the high Mo importance in symbiotic N fixation of legume, being an important cofactor of nitrogenase complex and xanthine oxidase, for N fixation and purine metabolism, respectively [9].

Pattanayak et al. [14] have done a field experiment on seed treatment of green gram at doses of 0.16 g/kg and 0.008 g/kg of seed for Mo and Co, respectively, results showed that the nodules number increased in a range of 122%-144%. However, Tenywa [19] reported that Mo at 390 g/ha and 780 g/ha applied to soybeans in acid soils did not significantly influence on the nodules number. In this study, Co application to RhMo treatment increased further nodules number in 107.6%, indicating some synergy between Co and Mo for enhancing the activity of rhizobia.

Inorganic N application increased grain N uptake (5.60 g/m^2), thus resulting in an increased total N removal (7.44 g/m^2). The addition of Mo to N fertilized plants drastically increased N partitioning in grains and the whole plant to 8.4 g/m^2 and 10.08 g/m^2, respectively. Again, the enhanced activity of plant enzymes, such as nitrate reductase that plays an important role in N plant metabolism [2], can be claimed as an explanation. As reported by Pessoa et al. [4], the enzymatic activity increased 16 times by adding 80 g Mo/ha to fertilized soybean crop with 60 kg N/ha, compared with the control. Similarly, Marschner [2] found it possible for logarithmic increasing of the nitrate reductase activity, providing 5 mg Mo per wheat plant versus control. But, considering N source, such as urea, Mo applying would be unnecessary. So, when Mo foliar applying was at 20 g Mo/ha to a common bean crop, the increasing of seed N content (8%) was not statistically significant [7].

When *Rhizobium etli* inoculated to bean, grain N and total N content increased at 3.4 g/m^2 and 4.58 g/m^2, respectively, while Mo adding to such treatment, N content increased at 4.8 g/m^2 and 5.77 g/m^2 in these variables, respectively (Table 2). These higher efficiencies could be explained by a higher activity of nitrogenase and xanthine oxidase enzymes in the dinitrogen reducing and purine catabolism, respectively [2]. Similarly, *Vigna radiata*, sowed in acid soil, increased proteins content by 2.02% and 10.7%, with application of 1.5 kg/ha Mo and 1 kg/ha Co, respectively [20]. And, chickpea genotypes, sowed in soil with pH 7.98, increased N content by 100.5%, when applied with 0.5 kg/ha Mo [21].

While, when Co supplies to beans treated with RhMo, seed N and total N content increased by 158%

and 140%, respectively. Similarly, in the mung bean those variables increased between 73%-75%. And, it was also showed by González et al. [5] that Co application to MoRh treatment increased those variables further (73%-90%). However, the increasing of such parameter was favored with individual application of these micronutrients, but not jointly [16].

The results are interesting that neither Mo nor Co applications affected N partitioning of shoots, which was significantly higher under N fertilized plants.

4. Conclusions

The addition of Mo to N-fertilized and *Rhizobium etli*-inoculated Canario Centenario beans increased grain yields. Mo increased also N removal by grains and total N plant uptake, and had a positive effect on grain size. The highest yields were obtained in inorganic N treatment. On the contrary, the inclusion of Co in bean fertilization did not result in a further yield increase. As expected, inoculation of *Rhizobium etli* increased the number and weight of nodules and N content, while in inorganic content treatment, the increase were smaller and the number of nodules decreased.

When Mo was included in the fertilization of common bean inoculated with *Rhizobium* (under a no-till system), it can result in yields comparable to those obtained by N-fertilized beans. Thus, application of Mo by seed impregnation to bean cv. Canario Centenario, with inorganic fertilization or inoculated with N fixing bacteria, can result in yield increases that range from 500 kg/ha to 800 kg/ha, which can be considered largely profitable for farmers in the central coast of Peru, characterized by alkaline and calcareous soils.

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