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Split application of molybdic fertilizer at the reproductive stage of common bean increases the molybdenum content in seed

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ABSTRACT. We evaluated the effects of split molybdenum (Mo) application at the reproductive stage of the common bean on seed Mo content (SMoC) and seed quality in two trials in the Zona da Mata region, Minas Gerais. Plants were sprayed with 100 or 600 g Mo ha⁻¹ at the V4 stage. The higher dose was also split into 100(V4)+500(R5), 100(V4)+500(R7), 100(V4)+250(R5)+250(R7), and 100(V4)+150(R5)+350(R7). SMoC from plants sprayed with 600 g (five treatments) was 3.7- or 62-fold higher than those sprayed with 100 g, with seeds from the former treatments exhibiting slightly poorer quality. Application of 100 g at V4 + 500 g at the reproductive stage (four treatments) increased SMoC 1.6- or 2.7-fold compared with SMoC from plants sprayed with 600 g at V4. Split application of 500 g with two sprays increased SMoC by 11% (p = 0.257) or 16% (p = 0.013) compared with one spray of 500 g. SMoC can be higher with Mo applied at R7 (pod formation) instead of at R5 (pre-flowering), without impairing seed quality. Thus, split Mo application between the R5 and R7 stages of the common bean saves molybdic fertilizer to produce Morich seeds, with only a slight decrease in seed quality.

Keywords: Phaseolus vulgaris, seed molybdenum, seed quality, seed vigor, seed germination.

Parcelamento da aplicação do adubo molíbdico na fase reprodutiva do feijão-comum aumenta o conteúdo de molibdênio na semente

RESUMO. Avaliamos os efeitos de parcelamentos da aplicação de molibdênio (Mo) na fase reprodutiva do feijão-comum no conteúdo de Mo na semente (CMoS) e na sua qualidade fisiológica em dois ensaios na Zona da Mata, Minas Gerais. Pulverizaram-se feijoeiros com 100 ou 600 g Mo ha¹ na fase V4. Esta última dose também foi parcelada: 100(V4)+500(R5), 100(V4)+500(R7), 100(V4)+250(R5)+250(R7) e 100(V4)+150(R5)+350(R7). O CMoS de plantas pulverizadas com 600 g (cinco tratamentos) foi 3,7 ou 62 vezes maior que o das pulverizadas com 100 g; sementes deste tratamento apresentaram qualidade ligeiramente melhor que as daqueles. Aplicação de 100 g em V4 + 500 g na fase reprodutiva (quatro tratamentos) aumentou 1,6 ou 2,7 vezes o CMoS comparativamente ao CMoS de plantas adubadas com 600 g em V4. Parcelamento de 500 g em duas pulverizações aumentou em 11% (p = 0,257) ou 16% (p = 0,013) o CMoS comparativamente a uma pulverização. O CMoS pode ser mais alto quando se pulveriza o Mo em R7 (formação de vagens) comparativamente a R5 (pré-floração), sem prejuízo à qualidade da semente. Logo, o parcelamento do Mo nessas duas fases do feijão-comum economiza fertilizante molíbdico para produzir sementes ricas em Mo, com ligeira redução da qualidade da semente.

Palavras-chave: Phaseolus vulgaris, molibdênio na semente, qualidade da semente, vigor da semente, germinação da semente.

Introduction

In the Zona da Mata region, Minas Gerais, Brazil, foliar application of molybdenum (Mo) at 70 to 100 g ha⁻¹ (Berger, Vieira, & Araújo, 1996; Pessoa, Ribeiro, Chagas, & Cassini, 2001) is recommended for the common bean (*Phaseolus vulgaris* L.) at stage V4 (third trifoliate leaf) to prevent or correct Mo deficiency (Berger et al., 1996). Mo can improve nitrogenase and nitrate reductase activities in the common bean (Pessoa et al., 2001), as both are enzymes linked to nitrogen (N) metabolism of

plants. Mo fertilization can also increase the common bean's resistance to diseases (Jesus et al., 2004; Polanco et al., 2014). Thus, yield can be enhanced up to 300% compared with common bean plants that were fertilized neither with Mo nor with an N side dressing (Amane, Vieira, Novais, & Araújo, 1999). Aside from these benefits, common bean seeds harvested from plants fertilized with Mo are heavier (Araújo, Araújo, Rocha, & Carneiro, 2009) and exhibit better quality (Leite, Araújo, Miranda, Vieira, & Pires, 2009) as well as greater protein concentration (Lopes et al., 2014).

530 Vieira et al.

Despite the advantages of using Mo fertilizer on the common bean, many farmers have no access to this technology, either because they are not familiar with it or because there is no molybdic fertilizer available locally. One low-cost strategy to solve this problem would be to provide farmers with Mo-rich seeds. Studies on the common bean have confirmed the efficacy of this approach (Vieira, Salgado, & Ferreira, 2005; Vieira, Paula Júnior, & Pires, 2011; Pacheco, Brito, Straliotto, Pérez, & Araújo, 2012; Almeida, Araújo, & Alves, 2013). Reducing the Mo fertilizer dose to produce Mo-rich seed can be achieved by determining the most appropriate growth stage at which to apply the fertilizer. Split Mo application during the vegetative stage of the common bean (Vieira et al., 2005) and the reproductive stage of the soybean (Campo, Araujo, & Hungria, 2009; Milani et al., 2010) may increase seed Mo content (SMoC) without impairing soybean seed quality (Milani et al., 2010). The efficacy of Mo application during the reproductive stage of the common bean has not been confirmed in controlled studies. In a study conducted in the Zona da Mata region, 200 to 300 g Mo ha⁻¹ sprayed at the R8 stage (pod filling) of the common bean reduced seed quality as evaluated by germination and accelerated aging tests (Vieira et al., 2015). Thus, Mo application at the common bean's reproductive stage should concentrate on R5 (pre-flowering), R6 (flowering) and R7 (pod formation) stages in trying to improve SMoC.

Our objective was to evaluate the effects of split Mo application at the reproductive stage of the common bean on Mo accumulation in seeds and on seed quality.

Material and methods

conducted Experiments were in Coimbra (20°49'45"S, 42°45'47"W, and at 716 m elevation) and Oratórios (20°24'14"S, 42°49'10"W, 486 m elevation) in a dystrophic Red-Yellow Argisol. Both districts belong to the Zona da Mata region, in the state of Minas Gerais, Brazil. The regional climate is classified as Cwa (subtropical warm with dry winter) by the Köppen classification. At the beginning of the experiment, the soil of Coimbra had the following characteristics at the depth of 0 to 20 cm: 560 g kg⁻¹ clay, 190 g kg⁻¹ silt, 250 g kg⁻¹ sand, pH_{H2O} (1:2.5) 4.7, 11 mg dm⁻³ P, 84 mg dm⁻³ K (Mehlich), and 0.5, 1.4 and 0.5 cmolc dm⁻³ of Al, Ca and Mg (extract with KCl mol L⁻¹), respectively. The soil of Oratórios had the following characteristics: 270 g kg⁻¹ clay, 150 g kg⁻¹ silt, 580 g kg⁻¹ sand, pH_{H2O} 5.8, 31 mg dm⁻³ P, 112 mg dm⁻³ K; and 0.0; 1.9 and 0.9 cmolc dm⁻³ of Al, Ca and Mg,

respectively. These soils have been cultivated for over 20 years with corn (*Zea mays* L.) or fallow system during the spring-summer season and the common bean or a fallow system during the fall-winter season. Soil was prepared with a disc followed by a heavy-disc harrow twice.

Seeds were sown in March 2010. Common bean plants were sprayed with 100 or 600 g Mo ha⁻¹ at the V4 stage. The dose of 100 g Mo ha⁻¹ at the V4 stage is recommended for the common bean to prevent or correct Mo deficiency in the soil of the Zona da Mata region (Berger et al., 1996). The dose of 600 g Mo ha⁻¹ was used to produce Mo-rich seeds of the common bean (Vieira, Paula Júnior, Carneiro, & Queiroz, 2014). The latter dose was also split as 100(V4) + 500(R5), 100(V4) + 500(R7), 100(V4) + 250(R5) + 250(R7), and 100(V4) + 150(R5)+350(R7). In these split applications, Mo applied at V4 (100 g ha⁻¹) also aimed to prevent or correct Mo deficiency in soil and to produce a high common bean yield. Sodium molybdate (Na₂MoO₄ 2H₂O) was dissolved in water and sprayed on plants with a handheld CO₂ sprayer delivering 290 L ha⁻¹ at 245 kPa using two hollow-cone XR 11002 nozzles spaced 0.5 m apart. A plastic sheet was used to avoid drift and possible contamination of the plots near the treated plot. Treatments were replicated four times in a randomized complete block design.

Each plot had four 3-m-long rows, spaced 0.5 m apart. The outer rows were used as borders. *Rhizobium* inoculation was not performed, but soils were naturally infested by native strains. Fifteen seeds m⁻¹ of the cultivar BRSMG Majestoso were sown. These seeds were poor in Mo $(0.05 \pm 0.02 \,\mu g$ Mo seed⁻¹). BRSMG Majestoso is a small-seeded genotype (Middle American gene pool) of "carioca" grain class (creamstriped) with indeterminate, prostrate, type III growth habit recommended for planting in the State of Minas Gerais. This cultivar is efficient at allocating foliarapplied Mo in the seeds (Vieira et al., 2014).

A basal fertilization of 25 kg N ha⁻¹, 38 kg P ha⁻¹, and 42 kg K ha⁻¹ was applied in the furrow during planting time. At the V4 stage, urea (100 kg ha⁻¹) was applied as side dressing, followed by sprinkler irrigation with 40 mm of water to minimize N volatilization. Foliar diseases were controlled preventively with the fungicide epoxiconazole (100 g ha⁻¹) at the V4 and R5 stages. In addition, in Oratórios, the fungicide fluazinam (0.75 kg ha⁻¹) was applied twice (R6 and R7 stages) for white mold [Sclerotinia sclerotiorum (Lib.) de Bary] control. Pests were controlled with three applications of clorpyriphos (0.4 L ha⁻¹) in Coimbra and with two applications of methamidophos (0.25 L ha-1) in Oratórios. Weeds were controlled with fomesafen at 0.25 kg ha⁻¹ and fluazifop-p-butil at 0.20 kg ha⁻¹. Overhead sprinkler irrigation was provided at 2-day intervals after sowing for good and uniform seedling emergence. Thereafter, irrigation supplemented rainfall so that the plots received at least 40 mm of water each week.

Data were obtained for final stand, seed yield, 100seed mass, seed Mo concentration and content, and seed quality. Yield and 100-seed mass were adjusted to 120 g ka⁻¹ moisture. Seed Mo concentrations were estimated using an atomic emission spectrometer with inductively coupled plasma (ICP-OES, Perkin-Elmer Optima 3300 DV) according to the procedures described by Vieira et al. (2014). Seed Mo content (SMoC) represents the accumulated Mo in one seed that will be available to the plant grown from this seed. For SMoC calculation, seed Mo concentration in μg g 1 was multiplied by the average mass of one dry seed (in g). Seed quality was evaluated by standard germination and accelerated aging tests according to methodology described by Pinto, Basso, Kulczynski, and Bellé (2014). In these two tests, a randomized complete block design with four replications was used.

Data were analyzed for homogeneity of variance with Barlett's test and for normality with Lilliefors's test using the software program "Sistema para Análises Estatísticas" (SAEG, version 9.1). In Coimbra, the percentage of seed germination in both standard germination and accelerated aging tests did not meet the homogeneity of variance and normality assumption. Thus, means were calculated using arcsine-square-root transformed data before analysis and were back transformed for data presentation. When the effect of treatment was

significant (p < 0.05), a set of five orthogonal contrasts (Table 1) was analyzed. The precise p values of these contrasts were reported. Values were reported as means \pm SD.

Results and discussion

Mo treatments did affect seed Mo concentration and content (p < 0.001) in both trials. However, Mo treatments did not affect final stand (p = 0.81, p = 0.30), yield (p = 0.84, p = 0.57), 100-seed mass (p = 0.56, p = 0.52), or standard seed germination (p = 0.29, p = 0.38) in Coimbra and Oratórios, respectively. The lack of significant effects of Mo treatments on yield was expected because Mo was sprayed at the V4 stage as recommended (Berger et al., 1996); in addition, N fertilizer was applied at sowing time (28 kg N ha⁻¹) and at side dressing (40 kg N ha⁻¹). In contrast with the present study, Vieira et al. (2015) found an eight percentage point decrease in standard seed germination when seeds fertilized with from plants Mo 90(V4) + 255(R6) + 255(R7) were compared with seeds from plants sprayed with 90 g Mo ha-1 at the V4 stage. Research on this topic, specifically related to the common bean, is preliminary. Future studies are required to more deeply explore the effects of split high doses of foliar-applied Mo at the reproductive stage on seed quality in different environmental conditions.

Table 1. Mean values (± SD) of seed molybdenum (Mo) concentration and content as well as germination after accelerated aging treatment in response to foliar application of 100 or 600 g Mo ha⁻¹ at V4 (third trifoliate leaf) or to split applications of 600 g Mo ha⁻¹ in the common bean in two district of the Zona da Mata region, Minas Gerais State, Brazil.

Dose of Mo (growth stage)	Seed Mo concentration (µg g ⁻¹)	Seed Mo content1 (µg seed-1)	Accelerated aging (%)
	corrigir		
		Coimbra	
1. 100 g ha ⁻¹ (V4)	0.2 ± 0.2	0.05 ± 0.05	96.5^{2}
2. 600 g ha ⁻¹ (V4)	8.9 ± 1.7	2.17 ± 0.38	95.5
3. $100(V4) + 500(R5)$	12.6 ± 1.3	3.15 ± 0.29	98.0
4. 100(V4) + 500(R7)	12.2 ± 1.4	2.96 ± 0.32	95.7
5. $100(V4) + 250(R5) + 250(R7)$	14.9 ± 1.6	3.60 ± 0.38	97.2
6. $100(V4) + 150(R5) + 350(R7)$	13.9 ± 0.9	3.47 ± 0.33	96.5
Contrast		p value	
1 vs. 2-6	< 0.0001	< 0.0001	-
2 vs. 3-6	< 0.0001	< 0.0001	-
3,4 vs. 5,6	0.0127	0.0128	-
3 vs. 4	0.4303	0.6458	-
5 vs. 6	0.5759	0.3341	-
		Oratórios	
1. 100 g ha ⁻¹ (V4)	8.2 ± 3.3	2.14 ± 0.86	79.0 ± 2.6
2. 600 g ha ⁻¹ (V4)	12.4 ± 4.8	3.34 ± 1.38	72.7 ± 2.6
$3.\ 100(V4) + 500(R5)$	26.0 ± 2.9	6.77 ± 0.58	75.8 ± 3.4
4. 100(V4) + 500(R7)	39.4 ± 8.5	10.23 ± 2.64	77.5 ± 4.0
5. $100(V4) + 250(R5) + 250(R7)$	33.6 ± 5.0	9.56 ± 2.09	70.9 ± 2.0
6. 100(V4) + 150(R5) + 350(R7)	34.3 ± 6.7	9.30 ± 1.87	74.8 ± 2.4
Contrast		p value	
1 vs. 2-6	< 0.0001	< 0.0001	0.0048
2 vs. 3-6	< 0.0001	< 0.0001	0.1670
3,4 vs. 5,6	0.6313	0.2574	0.0107
3 vs. 4	0.0021	0.0074	0.3631
5 vs. 6	0.8442	0.8210	0.0510

 1 For the seed Mo content calculation, the seed Mo concentration in μ g g $^{-1}$ was multiplied by the average mass of one dry seed (in g). 2 In Coimbra, data were transformed using arcsine-square-root before analysis, but untransformed means are presented.

532 Vieira et al.

On average, yield was 2246 kg ha⁻¹ (CV = 18%) and 2993 kg ha⁻¹ (CV = 8%), 100-seed mass was 30.7 and 28.2 g, and standard seed germination was 93 and 99% in Oratórios and Coimbra, respectively. Mo treatments affected seeds subjected to accelerated aging treatment in Oratórios (p = 0.005) but not in Coimbra (p = 0.870).

Mo in seeds harvested from plants sprayed with 600 g Mo ha⁻¹ were 3.7-fold (Oratórios) or 62-fold (Coimbra) higher than Mo in seeds harvested from plants sprayed with 100 g Mo ha⁻¹ (contrast 1 vs 2-6; Table 1). Soil pH was lower in Coimbra (4.7) than in Oratórios (5.8). The difference in pH of approximately one unit between these two soils means that solubility of MoO₄-2 was approximately 100-fold higher (Lindsay, 1979) in Oratórios than in Coimbra. This difference in soil pH helps to explain the higher SMoC in Oratórios. SMoC means obtained in this study with the split of 600 g Mo ha⁻¹ were somewhat higher compared to those obtained by Vieira et al. (2014) in the same area, with the same cultivar and also applying part of the Mo at the reproductive stage.

Application of 100 g Mo ha⁻¹ at V4 + 500 g Mo ha⁻¹ at the reproductive stage increased SMoC 1.6fold (Coimbra) or 2.7-fold (Oratórios) compared with SMoC of seeds from plants sprayed with 600 g Mo ha⁻¹ at V4 (contrast 2 vs. 3-6; Table 1). Vieira et al. (2005) obtained a 2.3-fold increase in SMoC when 1440 g Mo ha⁻¹ was split in four equal portions and applied at 17, 21, 27, and 32 days after emergence (vegetative stage), compared with SMoC of seeds harvested from plants that received one spray of 1440 g Mo ha⁻¹ at 21 days after emergence. Therefore, the present study reinforces the claim that split foliar application of Mo favors Mo accumulation in common bean seed and further indicates that the split is also effective at the reproductive stage. One advantage of spraying Mo at the reproductive stage is the greater amount of plant tissue available to intercept the Mo solution relative to plants at the vegetative stage. The average canopy closure of eight indeterminate type III growth habit genotypes of the common bean was 50-60% at V4, 70-85% at R5, and 80-87% at the R7 growth stage (Vieira et al., 2014).

Application of 100 g Mo ha⁻¹ at V4 + 500 g Mo ha⁻¹ split twice at the reproductive stage increased the SMoC by 16% (Coimbra) or 11% (Oratórios) compared with the SMoC from plants sprayed with 100 g Mo ha⁻¹ at V4 + 500 g Mo ha⁻¹ at the reproductive stage, but the difference was only significant in Coimbra (contrast 3,4 vs. 5,6; Table 1). Thus, splitting the Mo dose into two applications instead of one application during the reproductive

stage of the common bean might reduce the Mo dose to produce Mo-rich seed. According to Vieira et al. (2011), 3.6 μ g Mo seed⁻¹ would be sufficient to complement Mo uptake by irrigated common bean plants from a Mo-poor soil. Our results suggest that in Coimbra (pH = 4.7) this amount of Mo in seed is only achieved when 83% of the Mo dose (or 500 g Mo ha⁻¹) is split with two sprays during the reproductive stage. In Oratórios (pH = 5.8), however, the dose of Mo used to produce 3.6 μ g Mo seed⁻¹ could be much lower than 600 g Mo ha⁻¹ when 83% of the Mo dose is applied at the reproductive stage.

In Oratórios, the contrast 3 vs. 4 was highly significant for SMoC, suggesting that Mo applied at the R7 stage is more effective at increasing SMoC than Mo applied at the R5 stage. The higher canopy closure at R7 (80-87%) compared to that at R5 (70-85%) (Vieira et al., 2014) partially explains this result. The lack of significance of this contrast in Coimbra indicates that environmental factors may influence the results. The lack of significance of the contrast 5 vs. 6 in both sites indicates that a small increase in the dose of Mo applied at R7 with a concomitant reduction in the dose of Mo applied at R5 is ineffective to increase SMoC. The ability of a genotype to accumulate Mo in seed is associated with its capacity to re-transfer Mo from the roots, nodules and pod walls to the seed (Brodrick & Giller, 1991). Thus, foliar-applied Mo at the R8 stage (pod filling) could be a better approach to increase SMoC than foliar-applied Mo before this stage. However, foliar-applied Mo higher than 200 g ha-1 at the R8 stage may reduce seed quality (Vieira et al., 2015). In future studies, doses lower than 200 g Mo ha-1 applied at the R8 stage should be included as a treatment when trying to improve Mo accumulation in seed.

In Oratórios, two contrasts (1 vs. 2-6 and 3,4 vs. 5,6; Table 1) were highly significant for seed germination after accelerated aging treatment. The greater vigor of seeds from plants fertilized with 100 g Mo ha⁻¹ at V4 (79% of germination) compared with seeds from plants fertilized with 600 g Mo ha-1 (74% of germination) indicates that a high dose of Mo may slightly reduce seed vigor in particular environmental conditions. Vieira et al. (2015) also found some evidence that a high dose of Mo applied at the reproductive stage decreased seed quality. A four percentage point improvement in germination vigor with one application of 500 g Mo ha⁻¹ at the reproductive stage (plus 100 g Mo ha⁻¹ at V4) compared with split application with two sprays of 500 g Mo ha⁻¹ at the reproductive stage (plus 100 g Mo ha⁻¹ at V4) (contrast 3,4 vs. 5,6) is an unexpected result. Because the difference between these means

was occasional and small in magnitude it may be irrelevant considering field conditions.

Contrasts 3 vs. 4 and 5 vs. 6 (Table 1) were not significant in either site for standard seed germination and seed germination after accelerated aging treatment. These results might indicate that a high dose of foliar-applied Mo at the R7 stage does not affect seed vigor compared with foliar-applied Mo at the R5 stage.

Conclusion

A split high dose of foliar-applied Mo in the reproductive stage of the common bean favors the accumulation of Mo in seeds, with only a slight decrease in seed quality. This technique is an effective way of increasing seed Mo content, which leads to a reduction in the cost of producing Mo-rich seed.

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