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Growth, grain yield and calcium, potassium and magnesium accumulation in common bean plants as related to calcium nutrition

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ABSTRACT. The objective of this work was to evaluate the plant growth and grain yield characteristics and the accumulation of calcium, potassium and magnesium in the leaves and grains of common bean cultivars grown with different calcium concentrations in the nutrient solution. Two experiments were conducted with nutrient solutions in a soilless system in a greenhouse. In the first experiment, five calcium concentrations (1.10, 1.65, 2.20, 2.75 and 3.30 mmol L^{-1}) and three common bean cultivars (Iraí, BRS Expedito and Carioca) were evaluated. Higher calcium concentrations (2.20, 2.75, 3.30, 3.85, 4.40 and 4.95 mmol L^{-1}) and two common bean cultivars (BRS Expedito and Carioca) were tested in the second experiment. Dry mass of the shoot and root, grain yield and calcium accumulation in the leaves and grains increased linearly in common bean plants supplied with calcium concentrations between 2.20 and 4.95 mmol L^{-1}. The high calcium concentration did not reduce the accumulation of calcium, potassium and magnesium in the leaves and grains. Common bean plants grown with high calcium concentrations present higher dry mass of the shoot and root, high grain yield and high calcium concentration in the leaves and grains.

Keywords: dry mass, nutritional effect of calcium, *Phaseolus vulgaris*.

Crescimento, produtividade de grãos e acumulação de cálcio, potássio e magnésio em plantas de feijão relacionadas à nutrição com cálcio

RESUMO. O objetivo deste trabalho foi avaliar o crescimento da planta, os caracteres da produtividade de grãos e a acumulação de cálcio, potássio e magnésio nas folhas e grãos de cultivares de feijão em resposta à concentração de cálcio na solução nutritiva. Dois experimentos foram conduzidos em cultivo fora do solo com solução nutritiva e em ambiente protegido. No primeiro experimento foram avaliadas cinco concentrações de cálcio (1,10; 1,65; 2,20; 2,75 e 3,30 mmol L^{-1}) e três cultivares de feijão (Iraí, BRS Expedito e Carioca). Altas concentrações de cálcio foram testadas no segundo experimento: 2,20; 2,75; 3,30; 3,85; 4,40 e 4,95 mmol L^{-1} e duas cultivares de feijão (BRS Expedito e Carioca). A massa seca da parte aérea e das raízes, a produtividade de grãos e a acumulação de cálcio nas folhas e grãos foram aumentadas linearmente em plantas de feijão supridas com 2,20 a 4,95 mmol L^{-1} de cálcio. Altas concentrações de cálcio não reduziram a acumulação de cálcio, potássio e magnésio nas folhas e nos grãos. Plantas de feijão cultivadas com alta concentração de cálcio apresentam maior massa seca de parte aérea e de raízes, alta produtividade de grãos e alta concentração de cálcio nas folhas e nos grãos.

Palavras-chave: massa seca, efeito nutricional do cálcio, *Phaseolus vulgaris*.

Introduction

The availability of calcium to bean plants depends on its concentration in the soil solution and also on chemical soil properties, such as acidity and aluminum concentration (Quintana, Harrison, Palta, Nienhuis, & Kmiecik, 1999; Fageria, Stone, & Moreira, 2008; Ouertatani, Regaya, Ryan, & Gharbi, 2011). With low calcium availability, a reduction in plant height, leaf area and shoot and root growth in bean plants has been reported (Leal & Prado, 2008). Bean plants grown in a nutrient solution with depleted calcium concentrations presented signs of deficiency, such as necrosis of the root tips after 28 days and necrosis of the shoot apices after 30 days of growth, and these symptoms apparently caused a halt in growth, as indicated by the observed absence of xylem sap during harvesting (Schmitt et al., 2013). In fact, calcium serves vital physiological functions in plants, such as the organization of cell walls by calcium pectates, stabilization of membranes, pollen germination, pollen tube growth and elongation of the roots (Ge, Tian, & Russel, 2007; Gilliham et al., 2011).

Liming has been the main management practice used to neutralize soil acidity and aluminum and indirectly supplies calcium to crops. Liming effectively increased grain yield, the dry mass of the shoot and the number of pods and grains per plant in common beans (Fageria et al., 2008) and fava beans (Ouertatani et al., 2011). However, calcium is involved in complex reactions with other chemical elements present in the soil solution, and the soluble quantities available for plant uptake are difficult to estimate. The majority of research evaluating the response of bean plants to calcium was conducted in soil trials under field conditions, and the results have been contradictory for snap beans (Quintana et al., 1999) and common beans (Fageria et al., 2008). In fact, such results cannot separate the role of calcium as a soil neutralizer and its relations with other cations, such as potassium and magnesium, from its role as a nutrient for plant growth.

However, in a soilless system using a nutrient solution in a greenhouse it is possible to grow plants with the highest degree of control over factors such as nutrient uptake, water absorption, pH and salinity and also to isolate the effect of variations of other nutrients on plant growth and grain yield. Common bean plants supplied with high calcium concentrations in the nutrient solution showed an increase in the dry mass of the shoot and roots and higher calcium accumulation in the shoot and roots (Souza Junior, Nascimento, & Martinez, 1998; Silva, Moraes, & Souza, 2011). In these experiments, the plants were grown under controlled conditions and fed with nutrient solutions for periods ranging from 30 to 45 days.

Snap bean plants supplied with high calcium concentrations in the nutrient solution presented higher dry mass of the stem and leaves and high calcium accumulation in the leaves, followed by reproductive organs and stem (Pomper & Grusak, 2004; Schmitt et al., 2013). Previous studies also showed that an increase in calcium concentration in the nutrient solution resulted in an increase in fresh weigh, number of pods per plant, calcium accumulation in the pods and quality of snap beans (Favaro, Braga Neto, Takahashi, Miglioranza, & Ida, 2007). Cabot, Sibole, Barceló, and Poschenrieder (2009) observed that an increase in calcium in the nutrient solution decreased the potassium and magnesium concentrations in the leaves of snap bean plants. Snap beans are consumed in the form of green pods. In Brazil, common beans are preferred for consumption in the form of dry grains.

Common bean plants supplied with nutrient solutions with high calcium concentrations during the entire developmental cycle may have higher dry mass of the shoot and roots in the different phenological stages, high grain yield and high calcium, potassium and magnesium concentrations in the leaves and grains. These results were not found in the literature for dry beans. Furthermore, the portioning of calcium, potassium and magnesium in parts of the common bean plant needs to be further studied to be able to enhance the calcium concentration in the plant's edible parts by breeding. Therefore, the objective of this work was to evaluate the plant growth and grain yield characteristics and the accumulation of calcium, potassium and magnesium in the leaves and grains of common bean cultivars grown with different calcium concentrations in the nutrient solution.

Material and methods

Two experiments were performed in a closed soilless system inside a greenhouse at the Plant Science Department of the Federal University of Santa Maria, Santa Maria, Rio Grande do Sul State, Brazil (lat 29° 42' S, long 53° 49' W and 95 m altitude). The growing device used was described by Domingues, Ribeiro, Andriolo, Possobom, and Zemolin (2014). The plants were grown in 4 dm^3 black polypropylene pots filled with sand (1.2 to 2.4 mm gauge) previously washed with a 1% sodium hypochlorite solution.

The nutrient solutions were prepared according to the recommendations of Domingues et al. (2014). The electrical conductivity (EC) was adjusted so that a deviation of more than 10% from the initial value was recorded. For this, water or aliquots of new nutrient solution were added as required. The addition of KOH or 1 N H_2SO_4 into the nutrient solution was performed whenever necessary to maintain the pH between 5.5 and 6.5.

Experiment 1 was conducted from March to June 2011 in a completely randomized design with split plots and six repetitions. The main plots consisted of five calcium concentrations in the nutrient solution: 1.10, 1.65, 2.20, 2.75 and 3.30 mmol L^{-1}. The concentrations of other nutrients in each nutrient solution were adjusted to maintain the ionic equilibrium as follows, for total N, K^+, $H_2PO_4^-$, Ca^{++} and Mg^{++}, respectively, in mmol L^{-1}: 6.76, 4.50, 1.50, 1.00 and 1.00 in T1; 8.15, 5.00, 1.00, 1.00 and 1.00 in T2; 8.03, 5.00, 1.00, 1.00 and 1.75 in T3; 8.61, 5.70, 2.50, 1.00 and 1.75 in T4; 9.54, 5.25, 1.50, 1.50 and 2.00 in T5. The micronutrient quantities, in mg L^{-1}, were: 0.03 Mo, 0.42 B, 0.06 Cu, 0.50 Mn, 0.22 Zn and 1.00 Fe in all treatments. Three common bean cultivars, Iraí, BRS Expedito and Carioca, were grown in sub-plots. The Iraí cultivar comes from the Andean gene pool; it has cranberry-type grains (beige tegument with red stripes), determinate growth habit (type I) and early cycle. The BRS Expedito comes from the Middle American gene pool and has black commercial group grains, indeterminate growth habit with short guides (type II) and an intermediate cycle. The Carioca comes from the Middle American gene pool and has carioca-type grains (beige tegument with brown streaks), indeterminate growth habit with long guides (type III) and intermediate cycle. Twelve plants per treatment were used as the experimental units.

Three seeds per pot were sown on March 18, 2011, but only one plant per pot was kept after thinning at the first trifoliate leaf stage (V3). At the flowering stage (R6), six plants were collected, and the dry mass of the stems, leaves and shoot (stems + leaves) were determined after drying in an oven with forced air circulation (65 - 70ºC) until a constant mass was reached. At the maturation stage (R9), another six plants of each treatment were collected, and the number of pods and grains per plant, number of grains per pod and grain yield (g $plant^{-1}$ at 13% average moisture) were determined.

To determine the calcium concentration, 5 g samples of the dry mass of the stem and leaves at the R6 stage and the grains at the R9 stage were finely ground in an analytical knife mill to obtain particles smaller than 1 mm. The resulting flour was exposed to nitric-perchloric digestion, and the reading was performed using an atomic absorption spectrophotometer according to the method described by Domingues et al. (2014).

The second experiment was conducted from September to December 2011 to evaluate the effect of higher calcium concentrations than those used in Experiment 1 on plant growth, grain yield and the partitioning of calcium, potassium and magnesium. The same experimental design as the previous experiment was used. The main plots consisted of six calcium concentrations in the nutrient solution: 2.20 (T1), 2.75 (T2), 3.30 (T3), 3.85 (T4), 4.40 (T5) and 4.95 (T6) mmol L^{-1}, and two common bean cultivars, BRS Expedito and Carioca, were grown in sub-plots. The nutrient concentrations in T1, T2 and T3 were the same as in Experiment 1, and those of T4, T5 and T6 were as follows, for total N, K^+, $H_2PO_4^-$, Ca^{++} and Mg^{++}, respectively, in mmol L^{-1}: 9.67, 2.00, 1.00, 1.50 and 2.00 in T4; 11.06, 2.00, 1.00, 2.00 and 2.50 in T5; 12.44, 2.00, 1.00, 2.00 and 2.50 in T6. Micronutrients were supplied as in Experiment 1. Twenty-four plants per treatment were used as the experimental units.

Three seeds per pot were sown on September 15, 2011, but only one plant per pot was kept after thinning at the first trifoliate leaf stage (V3). In each developmental stage, third leaf trifoliate (V4), flowering (R6), pod filling (R8) and maturation (R9), six plants were collected from each treatment for the determination of the dry mass of the plant. The roots were washed in running water to remove the sand. The dry mass of the shoot and roots were determined after drying in an oven with forced air circulation (65 - 70ºC) until a constant mass was reached. At pod filling, the number of pods per plant and the dry mass of the pods were also evaluated. At maturation, the number of grains per plant was also counted, and the grain yield at 13% of average moisture was determined.

Samples of 5.0 g of the leaves at the V4, R6 and R8 stages and of the grains at the R9 stage of each treatment were finely ground to determine the nutrient concentration in the tissues, as described for Experiment 1. The determination of the calcium, potassium and magnesium concentrations was performed with an atomic absorption spectrophotometer using a wavelength of 422.70 nm for calcium, 766.50 nm for potassium and 202.50 nm for magnesium.

The data were subjected to variance analysis considering the completely randomized design with split plots and six repetitions, except for the nutrient concentration in the tissues, for which three repetitions were used. The F test (p value < 0.05) was used to test the hypotheses of the main effects and for the calcium concentration x cultivar interaction, considering all effects as fixed. When the effect of the calcium concentration and the calcium concentration x cultivar interaction were significant, regression analysis was done by the method of orthogonal polynomials, and the equation at the higher significant grade was retained. In cases in which the second grade equation fitted by regression analysis was also significant, the point of maximum (or minimum) technical efficiency was estimated by the equation ($X = -\hat{b}_1 / 2\hat{b}_2$), $\hat{b}_1$ and $\hat{b}_2$ being the coefficients of the first and second grade, respectively, obtained from the estimated equation of regression analysis. When the effect of the cultivars was significant, the comparison of means was carried out using the Tukey test (p = 0.05) in the first experiment and by the F test in the second experiment (p = 0.05). Statistical analyses were performed using the Microsoft® Office Excel spreadsheet and the Sigma Plot and Genes softwares.

Results and discussion

Experiment 1

In the variance analysis, a significant calcium concentration x cultivar interaction was observed only for the number of grains per pod ($p < 0.05$) (Table 1). However, the main effects (calcium concentration and cultivar) were significant for the dry mass of the stems, leaves and shoot, the number of pods and grains per plant, the grain yield and the calcium concentration in the grains. For the calcium concentration in the stems and leaves, a significant effect was observed only for the calcium concentration in the nutrient solution. A significant effect for the calcium concentration was previously described for the dry mass of the shoot (Favaro et al., 2007; Silva et al., 2011) and the number of pods per plant (Favaro et al., 2007) in bean plants supplied with calcium in the nutrient solution. Significant differences between snap bean cultivars for the calcium accumulation in the pods were previously reported by Pomper and Grusak (2004). Therefore, the increase in calcium concentration in the rooting medium was efficient to increase plant growth and grain yield in the bean plants, and the genetic variability among bean cultivars can be observed.

For the dry mass of the stems, leaves and shoot and the calcium concentration in the stems, significant effects were found for the calcium concentration in the nutrient solution, but the regressions of the 1st, 2nd and 3rd grades were not significant (Table 1). For these characteristics, there was no response, linear, quadratic or cubic, to the calcium concentration in the rooting medium. Other models showed no biological explanation and therefore were not presented. However, common bean plants grown with different calcium concentrations in the nutrient solution over a period of 45 days showed a quadratic response for the dry mass of the shoot (Silva et al., 2011). In the present work, the common bean plants were grown until the end of their cycle using different calcium concentrations, and this can justify the differences observed.

The number of pods and grains per plant and the grain yield increased due to the effect of the calcium concentration in the nutrient solution in the range between 1.1 and 3.3 mmol L^{-1} (Figures 1a, b and c). In snap beans, Favaro, Braga Neto, Takahashi, Miglioranza, and Ida (2007) reported that the number of green pods harvested 10-12 days after anthesis also increased with the calcium concentration in the nutrient solution. Similar effects were recorded by Domingues et al. (2014) for the number of pods and grains per plant, the number of grains per pod and the grain yield of common bean plants grown with a calcium concentration of 3.85 mmol L^{-1} in the nutrient solution.

Table 1. Variance and regression analysis for the dry mass of: stems (DMST, g), leaves (DML, g) and shoot (DMS, g); number of: pods per plant (NP), grains per plant (NG) and grains per pod (NGP); grain yield (yield, g plant^{-1}); calcium concentration in: stems (Ca in stems, g kg^{-1} of dry matter - DM), leaves (Ca in leaves, g kg^{-1} DM) and grains (Ca in grains, g kg^{-1} DM) evaluated in three common bean cultivars grown with five calcium concentrations in the nutrient solution in the first experiment.

Source of variation	Mean square					
	DF	DMST	DML	DMS	NP	NG
Concentration (A)	4	2.20^{*}	3.46^{*}	10.79^{*}	47.28^{*}	1318.76^{*}
Error A	25	0.79	0.84	2.85	7.51	190.36
Cultivar (B)	2	14.61^{*}	19.8^{*}	60.72^{*}	180.11^{*}	13149.9^{*}
A X B	8	1.39^{ns}	0.77^{ns}	3.77^{ns}	10.81^{ns}	463.31^{ns}
Error B	50	0.70	1.57	3.87	8.58	238.07
Grade 1	1	2.93^{ns}	4.60^{ns}	14.36^{ns}	63.04^{*}	1743.55^{*}
Grade 2	1	1.99^{ns}	3.23^{ns}	10.39^{ns}	57.05^{ns}	1624.90^{ns}
Grade 3	1	0.79^{ns}	1.08^{ns}	3.97^{ns}	5.60^{ns}	116.96^{ns}
Deviations	1	3.08	4.93	14.35	63.45	1719.57
Mean		4.25	4.99	9.25	10.47	46.76
C.V. (%)		19.62^{4}- 15.68^{5}	18.37^{4}-8.12^{5}	18.27^{4}-16.85^{5}	16.17^{4}-17.96^{5}	19.50^{4}-18.95^{5}

Source of variation	DF	NGP	Yield	DF	Ca in stems	Ca in leaves	Ca in grains
Concentration (A)	4	0.25^{ns}	174.54^{*}	4	11.18^{*}	43.37^{*}	0.08^{*}
Error A	25	0.21	18.03	10	1.17	12.19	0.43
Cultivar (B)	2	43.94^{*}	278.00^{*}	2	10.58^{ns}	52.22^{ns}	0.57^{*}
A X B	8	0.60^{*}	17.93^{ns}	8	0.97^{ns}	26.6^{ns}	0.53^{ns}
Error B	50	0.23	28.97	20	0.69	15.09	0.5
Grade 1	1	0.28^{1}-0.16^{2}-0.03^{3ns}	241.27^{*}	1	22.93^{ns}	66.45^{ns}	0.05^{*}
Grade 2	1	0.25^{1}-0.05^{2}-0.02^{3ns}	227.92^{ns}	1	9.47^{ns}	34.11^{*}	0.04^{ns}
Grade 3	1	0.02^{1}-0.05^{2}-0.01^{3ns}	2.95^{ns}	1	3.46^{ns}	21.99^{ns}	0.03^{ns}
Deviations	1	0.01^{1}-0.06^{2}-0.01^{3}	226.02	1	8.84	50.70	1.98
Mean		4.40	14.52		5.60	16.16	0.95
C.V. (%)		10.43^{4}-8.26^{5}	12.65^{4}-17.06^{5}		19.33^{4}-14.85^{5}	11.60^{4}-14.03^{5}	14.36^{4}-11.75^{5}

*Significant at 5% probability by F test. ^{ns}not significant. [1]Value corresponding to BRS Expedito cultivar. [2]Value corresponding to Carioca cultivar. [3]Value corresponding to Iraí cultivar. [4]Coefficient of variation of the main plot. [5]Coefficient of variation of the subplot.

These results might be due to the effect of calcium on pollen germination and the growth of the pollen tube, leading to higher fertility (Ge et al., 2007) and thereby leading to an increased number of pods and grains per plant and higher grain yield (Figure 1a, b and c). However, although a linear relationship was fitted for these characteristics, there should be a maximum calcium concentration above which this effect will no longer be recorded.

The highest calcium concentration in the leaves was reached at a calcium concentration of 2.64 mmol L^{-1} in the nutrient solution (point of maximum technical efficiency) (Figure 1d). Snap bean plants were responsive to increases in the calcium concentration in the nutrient solution, and the highest calcium accumulation was observed in the leaves (Favaro et al., 2007; Cabot, Sibole, Barceló, & Poschenrieder, 2009; Schmitt et al., 2013).

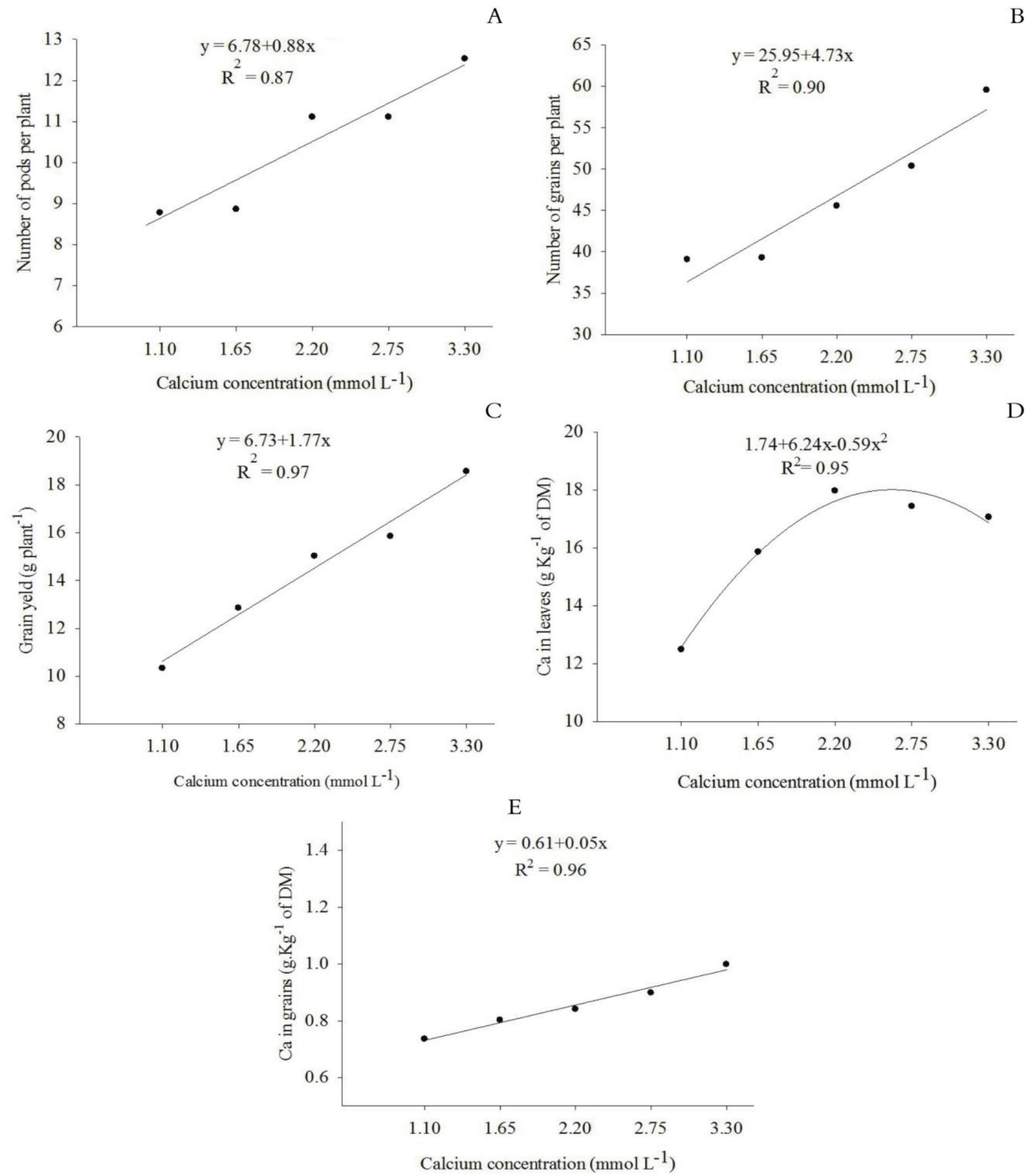

Figure 1. Number of pods per plant; a), number of grains per plant; b), grain yield (g $plant^{-1}$); c), calcium concentration in leaves (Ca in leaves, g kg^{-1} of dry matter - DM); d) and calcium concentration in grains (Ca in grains, g kg^{-1} DM); e), of three common bean cultivars grown with five calcium concentrations in the nutrient solution in the first experiment.

In the present work, the decrease at the upper calcium concentrations in the nutrient solution may have been a simultaneous effect of the reduction in the water flow in the plant and the higher electrical conductivity of the nutrient solutions. This experiment was conducted in autumn, and the plants reached full leaf area growth in later autumn, when the atmospheric water demand and transpiration were lower. When plant water demand is low, the plants might be more sensitive to salinity. This hypothesis is supported by the results in the literature reporting that the highest calcium accumulation in the shoot of common bean plants was obtained at a calcium concentration of 1.40 mmol L^{-1} in the nutrient solution (Souza Junior et al., 1998).

The calcium concentration in the grains increased 35% and fitted a linear regression in the range of treatments (Figure 1e). In snap beans grown in soil, it has been reported that calcium fertilization was not effective to increase the calcium concentration in immature pods (Quintana et al., 1999). These differences may also be related to the water flow in the plant during its growing period. In indeterminate growth snap bean plants, the leaves and pods grow simultaneously and may compete for water during periods of high transpiration demand by the plants. At the final developmental stages of determinate growth in plants, nutrients are remobilized from the vegetative organs to the grains, and the nutrient concentration in the grains can increase. In the present experiment, the calcium uptake, transport and accumulation in the plant organs might also be enhanced because this ion was in a readily available form in the nutrient solution. This is not the case in plants grown in soil, where the uptake of calcium might be depressed by interactions with other nutrients and/or chemical elements dissolved in the soil solution.

The common bean cultivars differed in their plant growth and grain yield characteristics (Table 2). The BRS Expedito and Carioca cultivars showed higher dry mass of the stems, leaves and shoot and higher number of pods and grains per plant and number of grains per pod than the Iraí cultivar. This is explained by the fact that the BRS Expedito and Carioca cultivars, which are of indeterminate growth habit, had vigorous shoot growth, resulting in shading of the Iraí plants. Therefore, the Iraí cultivar showed the lowest values in dry mass of the plant and grain yield components. The highest grain yield was obtained by the Carioca cultivar.

Table 2. Mean values for the dry mass of stems (DMST, g), dry mass of leaves (DML, g), dry mass of shoot (DMS, g), number of pods per plant (NP), number of grains per plant (NG), number of grains per pod (NGP), grain yield (yield, g $plant^{-1}$) and calcium concentration in grains (Ca in grains, g kg^{-1} of dry matter - DM) evaluated in three common bean cultivars grown with five calcium concentrations in the nutrient solution in the first experiment.

Cultivar	DMST	DML	DMS	NP	NG	NGP	Yield	Ca in grãos
BRS Expedito	4.84 a*	5.11 a	9.94 a	10.88 a	54.57 a	5.12 a	14.27 b	1.27 a
Carioca	4.45 a	5.75 a	10.19 a	12.70 a	62.68 a	5.10 a	17.69 a	0.93 ab
Iraí	348 b	4.13 b	7.62 b	7.85 b	23.05 b	3.01 b	11.62 b	0.63 b
Mean	4.25	4.99	9.25	10.47	46.76	4.40	14.52	0.95
C.V.%	15.68	8.12	16.85	17.96	18.95	8.26	17.06	11.75

*Means followed by the same letter in the column do not differ significantly ($p = 0.05$) by Tukey's test. C.V.%: Coefficient of variation.

The calcium accumulation in the grains differed among the cultivars, with BRS Expedito differing significantly from Iraí (Table 2). BRS Expedito is a cultivar in use in Brazil featuring black grains with a high calcium concentration in the grains (Jost et al., 2013). This cultivar showed high calcium accumulation in the grains when grown with low and high calcium availability in the nutrient solution (Domingues, Ribeiro, Andriolo, Possobom, & Zemolin, 2014). These results suggest that the common bean cultivars with black grains such as BRS Expedito might be higher accumulators of calcium in the grains. Differences in calcium accumulation in the pods of snap bean cultivars have also been reported in the literature (Pomper & Grusak, 2004; Quintana et al., 1999). According to Pomper and Grusak (2004), snap bean plants of the Labrador cultivar, which exhibit low pod calcium concentration, showed higher whole-plant water uptake, and this led to a dilution of the calcium concentration in the xylem stream, and thus less total calcium was transported to the developing pods.

Experiment 2

A significant calcium concentration x cultivar interaction ($p < 0.05$) was observed for the potassium concentration in the leaves at R8 and for the magnesium concentration in the grains at R9 (Table 3). For these characteristics, the response of the common bean cultivars differed depending on the calcium concentration supplied in the nutrient solution.

Significant effects of the calcium concentration were observed on the dry mass of the shoot at R6, R8 and R9, dry mass of the roots at R6 and R9, dry mass of the pods at R8, grain yield, calcium concentration in the leaves at R8 and in the grains at R9, potassium concentration in the grains at R9 and magnesium concentration in the leaves at R8. Thus, the calcium availability in the rooting medium affected these characteristics. A significant effect of the cultivars ($p < 0.05$) was observed for the dry mass of the roots at R6 and R9, number of pods per plant at R8, dry mass of the pods at R8, potassium concentration in the grains at R9 and magnesium concentration in the leaves at R8. The existence of significant differences among the cultivars for these characteristics allows the selection of superior common bean cultivars.

In the second experiment the dry mass of the shoot at R6 and R8 and the dry mass of the root at R6 showed significant response to the increase in the calcium concentration in the nutrient solution (Figures 2a, b and c). Shoot growth increased at calcium concentrations in the nutrient solution above 3.30 mmol L^{-1} in both plant phenological stages (Figures 2a and c), while root growth increased linearly at R6 in the range between 2.20 and 4.95 mmol L^{-1} (Figure 2b). Silva, Moraes, and Souza (2011) observed that the growth of common bean plants at 45 days after planting was reduced by a calcium concentration of 0.46 mmol L^{-1} in the nutrient solution. In later plant stages, the calcium requirements may be higher, and reduction in growth at higher calcium concentrations could not be excluded. High root growth under high calcium availability has also been reported in the literature for bean plants (Souza Junior et al., 1998; Favaro et al., 2007; Silva et al., 2011). In the present work, the quite large range of concentrations used as treatments produced a surprisingly linear response without a saturation point and without any signs of toxicity observed in the plants.

Table 3. Variance and regression analysis for the dry mass of the: shoot at third trifoliate leaf (DMS V4, g), roots at third trifoliate leaf (DMR V4, g), shoot at flowering (DMS R6, g), roots at flowering (DMR R6, g), shoot at pod filling (DMS R8, g) and roots at pod filling (DMR R8, g); number of pods at pod filling (NP R8); dry mass of: pods at pod filling (DMP R8, g), shoot at maturity (DMS R9, g) and roots at maturity (DMR R9, g); number of grains per plant at maturity (NG R9), grain yield (yield, g $plant^{-1}$); calcium in the: leaves at third trifoliate leaf (Ca L V4, g kg^{-1} of dry matter - DM), leaves at flowering (Ca L R6, g kg^{-1} DM), leaves at pod filling (Ca L R8, g kg^{-1} DM) and grain at maturity (Ca G R9, g kg^{-1} DM); potassium in the: leaves at pod filling (K L R8, g kg^{-1} DM) and grains at maturity (K G R9, g kg^{-1} DM); magnesium in the: leaves at pod filling (Mg L R8, g kg^{-1} DM) and grains at maturity (Mg G R9, g kg^{-1} DM) evaluated in two common bean cultivars grown with six calcium concentrations in the nutrient solution in the second experiment.

Source of variation	Mean square							
	DF	DMS V4	DMR V4	DMS R6	DMR R6	DMS R8	DMR R8	NP R8
Concentration (A)	5	0.38^{ns}	0.03^{ns}	27.92^{*}	1.99^{*}	56.04^{*}	0.54^{ns}	19.55^{ns}
Error A[1]	30	0.63	0.02	5.36	0.59	10.93	0.49	9.92
Cultivar (B)	1	0.45^{ns}	0.02^{ns}	8.00^{ns}	21.23^{*}	0.11^{ns}	0.10^{ns}	84.5^{*}
A X B	5	0.39^{ns}	0.005^{ns}	6.16^{ns}	0.47^{ns}	5.13^{ns}	0.21^{ns}	2.26^{ns}
Error B[2]	30	0.21	0.009	4.77	0.43	8.67	0.54	8.70
Grade 1	1	0.85^{ns}	0.006^{ns}	41.99^{*}	4.05^{*}	112.47^{*}	0.69^{ns}	28.00^{*}
Grade 2	1	0.94^{ns}	0.002^{ns}	25.45^{*}	0.77^{ns}	23.73^{ns}	0.59^{ns}	34.83^{*}
Grade 3	1	0.10^{ns}	0.001^{ns}	2.40^{ns}	0.18^{ns}	3.84^{ns}	0.08^{ns}	6.03^{ns}
Deviations	1	0.83	0.14	69.80	4.99	140.11	1.35	28.89
Mean		1.30	0.14	16.85	2.79	21.03	4.33	19.13
C.V. (%)		19.31[3]-11.31[4]	22.05[3]-21.50[4]	17.18[3]-12.96[4]	13.74[3]-23.64[4]	15.72[3]-14.00[4]	16.20[3]-17.06[4]	16.46[3]-15.41[4]

Source of variation	DF	DMP R8	DMS R9	DMR R9	NG R9	Yield	DF	Ca L V4
Concentration (A)	5	30.09^{*}	66.41^{*}	1.28^{*}	287.42^{ns}	39.38^{*}	5	10.54^{ns}
Error A[1]	30	9.94	22.21	0.53	138.62	8.46	12	5.40^{*}
Cultivar (B)	1	93.16^{*}	34.03^{ns}	34.72^{*}	320.88^{ns}	6.90^{ns}	1	17.05^{ns}
A X B	5	7.12^{ns}	77.09^{ns}	3.10^{ns}	306.02^{ns}	11.06^{ns}	5	1.57^{ns}
Error B[2]	30	8.12	25.67	0.71	230.16	10.61	12	6.01
Grade 1	1	39.08^{*}	80.21^{ns}	2.14^{ns}	366.42^{ns}	55.70^{*}	1	18.69^{*}
Grade 2	1	51.62^{*}	66.42^{ns}	1.05^{ns}	128.51^{ns}	14.33^{ns}	1	9.77^{*}
Grade 3	1	4.54^{ns}	22.97^{ns}	0.83^{ns}	223.68^{ns}	7.70^{ns}	1	8.92^{ns}
Deviations	1	55.24	106.04	2.40	718.56	98.46	1	15.32
Mean		17.92	30.69	5.18	28.27	24.51		15.66
C.V. (%)[3]		17.59[3]-15.90[4]	15.35[3]-16.51[4]	14.05[3]-16.29[4]	13.01[3]-16.77[4]	11.87[3]-13.29[4]		14.84[3]-15.64[4]

Source of variation	DF	Ca L R6	Ca L R8	Ca G R9	K L R8	K G R9	Mg L R8	Mg G R9
Concentration (A)	5	18.67^{ns}	96.71^{*}	0.56^{*}	959.95^{*}	21.91^{*}	3.86^{*}	1.14^{*}
Error A[1]	12	7.18	3.92	0.07	13.72	2.13	0.27	0.41
Cultivar (B)	1	3.82^{ns}	16.88^{ns}	0.21^{ns}	1887.7^{*}	136.11^{*}	14.68^{*}	2.68^{*}
A X B	5	12.41^{ns}	9.25^{ns}	0.02^{ns}	150.09^{*}	5.41^{ns}	0.62^{ns}	0.47^{*}
Error B[2]	12	4.13	4.12	0.01	8.52	8.79	0.23	0.48
Grade 1	1	14.32^{*}	175.07^{*}	0.15^{*}	410.5[6]-81.6[7ns]	75.30^{ns}	5.65^{ns}	0.57- 0.58[*5]
Grade 2	1	54.45^{ns}	63.52^{ns}	0.54^{ns}	245.9[6]-590.7[7ns]	23.40^{ns}	2.93^{ns}	0.64- 0.50^{ns}
Grade 3	1	5.29^{ns}	86.62^{ns}	0.01^{ns}	222.8[6]-161.3[7n.s.]	0.38^{ns}	2.47^{ns}	0.42- 0.72^{ns}
Deviations	1	1.34	158.41	0.09	151.7[6]-221.7[7]	10.47	8.28	1.22- 1.46
Mean		19.58	32.39	1.51	50.83	31.44	9.42	2.10
C.V. (%)[3]		13.68[3]-10.38[4]	6.11[3]-6.27[4]	5.76[3]-6.81[4]	7.28[3]-5.74[4]	4.64[3]-9.43[4]	5.55[3]-5.16[4]	9.62[3]-10.42[4]

*Significant at 5% probability by F test.^{ns}not significant. [1]Error A: main plot error. [2]Error B = subplot error. [3]Variation coefficient of main plot. [4]Coefficient of variation of the subplot. [5]Significant only for BRS Expedito cultivar. [6]Value for BRS Expedito cultivar. [7]Value for Carioca cultivar.

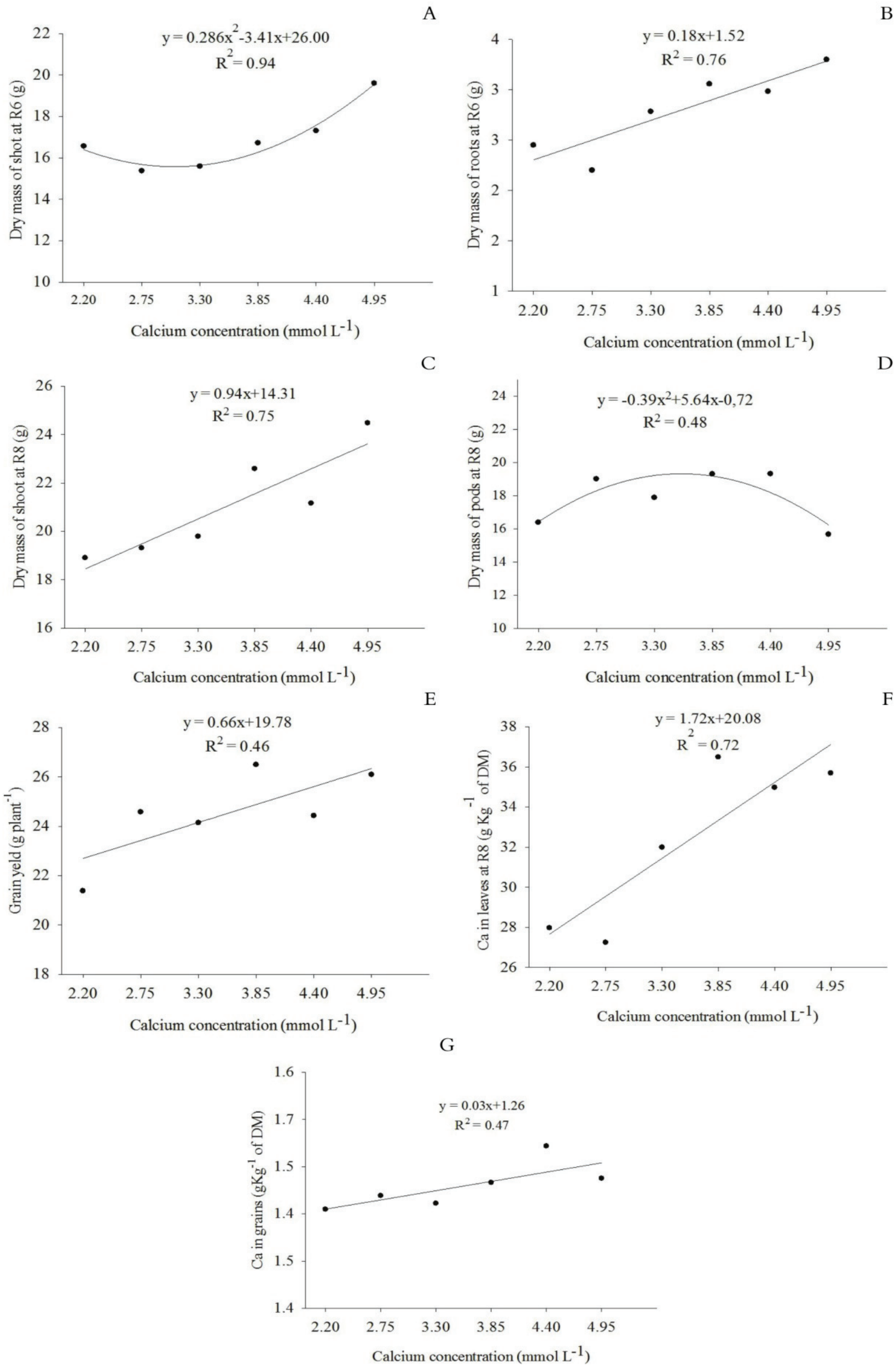

Figure 2. Dry mass of: shoot at flowering (R6) (g); a), roots at flowering (R6) (g); b), shoot at pod filling (R8) (g); c), and pods at pod filling (R8) (g); d), grain yield (g $plant^{-1}$); e), calcium concentration in the leaves at pod filling (Ca in leaves at R8, g kg^{-1} of dry matter - DM); f) and in the grains (Ca in grains, g kg^{-1} DM); g), of two common bean cultivars grown with six calcium concentrations in the nutrient solution in the second experiment.

In Experiment 1, the number of pods per plant increased with the increasing calcium concentration in the rooting medium (Figure 1a), but this effect was not significant in Experiment 2 (Table 3). Perhaps the genetically determined potential number was reached. The dry mass of the pods at R8 increased until a calcium concentration of 3.55 mmol L^{-1} in the nutrient solution (point of maximum technical efficiency) (Figure 2d). When supplying snap bean plants with calcium in the rooting medium, Favaro et al. (2007) also observed an improvement in the dry mass of the green pods. Nevertheless, the grain yield increased linearly (Figure 2e), confirming the tendency fitted in the first experiment (Figure 1c). In bean plant growth in soil, it has been reported that calcium fertilization was effective to increase the grain yield, the number of pods and the number of grains per plant in common beans (Fageria et al., 2008) and fava beans (Ouertatani et al., 2011). The increase in grain yield with calcium fertilization was mainly associated with an increase in the dry mass of the shoot (Fageria et al., 2008)

The leaves of the common bean plants were large accumulators of calcium at the phenological stages of third leaf trifoliate (15.66 g kg^{-1} of DM), flowering (19.58 g kg^{-1} of DM) and pod filling (32.39 g kg^{-1} of DM), followed by the grains at maturity (1.51 g kg^{-1} of DM) (Table 3). In snap bean cultivars, Pomper and Grusak (2004) also observed that the calcium concentration in the leaves differed according to the phenological stage, and the maximum value was found at the pod filling stage. Schmitt et al. (2013) supplied snap bean plants with calcium in the nutrient solution for six weeks and verified that the calcium accumulation was higher in the leaves (64%), followed by the reproductive organs (16%), stem (15%) and roots (5%). Understanding the partitioning of calcium in common bean plants in different phenological stages is very important to increase the calcium concentration in the edible parts of the plant.

The calcium concentration increased linearly in the leaves at R8 (Figure 2f) and also in the grains at R9 (Figure 2g). In the leaves, the calcium concentration averaged 28%, ranging from 27.98 g kg^{-1} of DM at the lowest to 35.68 g kg^{-1} DM at the highest calcium concentration in the nutrient solution. Jáuregui-Zùniga, Ferrer, Calderón, Munoz, and Moreno (2005) observed a positive correlation between the calcium concentration in the rooting medium of bean plants and calcium oxalate crystal formation in the leaves. In the present study, the leaves of common bean plants were found to be large accumulators of calcium (Table 3, Figure 2f). Therefore, the calcium bioavailability needs to be better assessed in the leaves of common bean plants grown with high calcium concentrations in the rooting medium.

In the grains at R9, the calcium concentration increased to 19%, from 1.41 to 1.68 g kg^{-1} DM in the same concentration range of the nutrient solution. These calcium values were higher than those obtained in the grains of 29 Andean lines with different tegument colors, cultivated in Brazil, in the United States and in Colombia (Akond, Crawford, Berthold, Talukder, & Hossain, 2011), and were considered high for common bean lines according to Ribeiro, Domingues, Zemolin, and Possobom (2013), Ribeiro et al. (2014) and Ribeiro, Jost, Maziero, Storck, and Rosa (2014). However, values for the calcium concentration in the grains varying from 0.7 to 1.4 g kg^{-1} of DM were found in 10 inbred lines of common beans grown with high calcium availability in the nutrient solution (3.85 mmol L^{-1}) by Domingues et al. (2014). Thus, in the present study, increasing the calcium concentration in the nutrient solution increased the calcium concentration in common bean grains. These data are very important because it indicates that improvement of the nutritional quality of common bean grains can be achieved by calcium management in the rooting medium.

The average calcium concentration in the leaves was 21% higher than in the grains, confirming data reported in the literature showing that the leaves of common beans and snap beans are strong calcium accumulators (Souza Junior et al., 1998; Pomper & Grusak, 2004; Favaro et al., 2007; Schmitt et al., 2013). Although no references in the literature were found regarding the use of common bean leaves as a source of calcium in human or husbandry diets, this might be a matter of interest in the future. In the present work, the calcium effect as a nutrient "per se" was evaluated because the effects of aluminum and/or other ions affecting soil chemical properties were excluded. In fact, such interactions may be the cause of the data presented in the literature showing that when calcium was applied in the soil, increases in the calcium concentration in the immature pods of snap beans have been recorded (Quintana et al., 1999).

In the present work, the ionic concentration of the nutrient solution had to be adjusted to obtain different calcium concentrations using soluble salts. As a consequence, the potassium was reduced, while the magnesium and nitrogen concentrations were increased to counterbalance the higher calcium concentrations. No disturbances in the K:Ca:Mg cationic ratio or salinity effects reducing plant growth and grain yield were recorded, and no effects on the potassium and magnesium concentrations in the leaves at R8 and the grains at R9, whether linear, quadratic or cubic, were observed (Table 3). Cabot et al. (2009) observed that an increase in calcium concentration in the nutrient solution in the presence of sodium chloride decreased the potassium and magnesium concentrations in the leaves of snap bean plants grown for 15 days. Providing potassium to snap bean plants grown in pots with soil produced a significant decrease in the calcium concentration in the leaves and stem and decreased the magnesium concentration in the pods and stem (Tüma, Skalicky, Tümová, Bláhová, & Rosülková, 2004). In the present study, all the nutrients in the nutrient solution were available for uptake by the plants, and it is unlikely that the concentrations in the plant organs were artifacts as a consequence of antagonisms and/or interactions among them.

The BRS Expedito cultivar showed higher dry mass of the roots at R6 and R9 and dry mass of the pods at R8 (Table 4). However, the Carioca cultivar had the higher number of pods per plant at R8. The identification of common bean cultivars with differential response in the growth and grain yield characteristics to different calcium concentrations in the nutrient solution is of great importance to common bean breeding because this allows the identification of a mechanism that gives the plant an ability to absorb and use calcium and thereby enables the selection of cultivars that are more efficient in calcium use.

Table 4. Mean values for the dry mass of roots at flowering (DMR R6, g), number of pods at pod filling (NP R8), dry mass of pods at pod filling (DMP R8, g) and dry mass of the root at maturation (DMR R9, g) evaluated in two common bean cultivars grown with six calcium concentrations in the nutrient solution in the second experiment.

Cultivar	DMR R6	NP R8	DMP R8	DMR R9
BRS Expedito	3.51*	17.44*	18.34*	6.24*
Carioca	2.11	21.19	15.82	4.19
Mean	2.81	19.35	17.08	5.21
C.V. (%)	23.64	15.41	15.90	16.29

*Significant at 5% by F test.ns = not significant. C.V. (%): coefficient of variation.

The results of the present work are original and can contribute to deeper knowledge of the mineral nutrition of the common bean crop. First, no data were found in the literature reporting the results of common bean plants supplied by nutrient solutions with different calcium concentrations on plant growth, grain yield and the accumulation of calcium, potassium and magnesium in parts of the plant at different phenological stages during the entire developmental cycle. Second, in this work, the effect of calcium as a nutrient was evaluated in conditions with no toxic effect of aluminum and/or chemical interactions present in the soils that negatively affect plant growth and grain yield. Third, calcium fertilization is a practice that could be previewed to increase the grain yield of this crop. This is an important conclusion of this research because current recommendations aim only to correct the pH and to neutralize the aluminum in the soil. Nevertheless, to reach this goal, more research in soil conditions must be done. The role of liming as a soil corrective had to be separate from the calcium nutrition effects. In the present research, the linear effects of calcium on plant growth, grain yield and calcium accumulation in the organs were recorded. This is not a realistic response of plant growth processes, which are mainly of the saturation type. Experiments in soil conditions should be performed to determine the maximum calcium fertilization rates to be used for this crop.

Conclusion

The dry mass of the shoot and root, the grain yield and the calcium accumulation in the leaves and grains increase linearly in common bean plants supplied with nutrient solutions with calcium concentrations between 2.20 and 4.95 mmol L^{-1}. The high calcium concentrations do not reduce the plant growth and grain yield or the accumulation of calcium, potassium and magnesium in the leaves and grains. Common bean plants grown with high calcium concentrations in the nutrient solution present higher dry mass of the shoot and root, high grain yield and high calcium concentration in the leaves and grains.

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