

# Comparison of Soil and Genotypic Effects on Calcium Concentration of Snap Bean Pods

Edison Miglioranza

Departamento de Agronomia, Universidade de Londrina, Londrina-PR, 86051, Brazil

Phillip Barak

Department of Soil Science, University of Wisconsin-Madison, Madison, WI 53706

Kenneth Kmiecik and James Nienhuis<sup>1</sup>

Department of Horticulture, University of Wisconsin-Madison, Madison, WI 53706

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**Abstract.** Soils were fertilized with gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) at rates up to 4 t·ha<sup>-1</sup>, and  $\text{Ca}^{2+}$  concentrations in pods of 12 snap bean (*Phaseolus vulgaris* L.) cultivars were determined, with the intention of improving snap beans as a source of  $\text{Ca}^{2+}$  for human nutrition. The addition of gypsum to the soil did not affect the  $\text{Ca}^{2+}$  concentration of pods, even though  $\text{Ca}^{2+}$  in the soil solution increased from 4 to 15 mmol·L<sup>-1</sup>. Calcium concentrations of pods of the various snap bean cultivars ranged from 4.1 to 5.7 mg·g<sup>-1</sup> dry mass. 'Top Crop', 'Astrel', 'Tenderlake', and 'True Blue' had the highest  $\text{Ca}^{2+}$  concentration in the pods and 'Labrador' and 'Roma II' had the lowest. The results suggest that factors other than  $\text{Ca}^{2+}$  supply influenced the  $\text{Ca}^{2+}$  concentration of the snap bean pod. Therefore, increased  $\text{Ca}^{2+}$  concentration of pods may be better achieved through breeding and selection rather than  $\text{Ca}^{2+}$  fertilization when  $\text{Ca}^{2+}$  levels in soil are sufficient.

Humans need ≈1200 mg of calcium ( $\text{Ca}^{2+}$ ) daily in adolescence (National Research Council, 1989) to ensure maximal bone accretion and to potentially prevent osteoporosis later in life (Johnston et al., 1992). Osteoporosis affects ≈25% of American women and ≈7% of men over 60 years old (Herbert et al., 1990).

Dairy products represent the main source of nutritional  $\text{Ca}^{2+}$  in the United States, amounting to 55%, 46%, and 42% of the total  $\text{Ca}^{2+}$  intake in adolescents, young adult females, and 60- to 65-year-old women, respectively (Pennington and Young, 1991). Green leafy vegetables provide a relatively good source of  $\text{Ca}^{2+}$  (Macrae et al., 1993). Among 39 major fruits and vegetables analyzed for nutritional value, snap beans ranked third for  $\text{Ca}^{2+}$  concentration (Stevens, 1974). Because ≈30% of teenage boys and 25% of teenage girls are likely to include snap beans in their diet (Pao

et al., 1982), snap beans are a potentially significant source of dietary  $\text{Ca}^{2+}$ . Calcium from low oxalic acid vegetable greens, such as snap bean, exhibits adsorbability equal to or better than  $\text{Ca}^{2+}$  in milk (Heaney et al., 1993). A 120-g serving (1/2 cup) of fresh snap beans can provide 60 mg of  $\text{Ca}^{2+}$ , or ≈5% of daily  $\text{Ca}^{2+}$  requirement of an adolescent. Thus, although vegetables are significant sources of  $\text{Ca}^{2+}$ , they could clearly benefit from enhancement in their  $\text{Ca}^{2+}$  concentration.

Significant genetic differences in pod  $\text{Ca}^{2+}$  concentration exist among snap bean  $S_1$  families, and the heritability of pod  $\text{Ca}^{2+}$  concentration was estimated at  $0.50 \pm 0.03$  (Quintana et al., 1996). Although pod  $\text{Ca}^{2+}$  concentrations differ significantly among snap bean cultivars, the relative magnitudes of genotypic, environmental, and genotype × environmental interaction effects on snap bean pod Ca concentration have not previously been reported.

The objectives of this study were to 1) determine if snap bean cultivars provided with a higher soil  $\text{Ca}^{2+}$  supply would have a corresponding higher  $\text{Ca}^{2+}$  concentration in the pod, and 2) determine if the effect of soil  $\text{Ca}^{2+}$  supply was consistent across various adapted snap bean cultivars.

## Materials and Methods

The beans were seeded 27 June 1994 in a Plainfield loamy sand (Typic Udipsamment, sandy, mixed mesic) with 1% organic matter and pH 6.6, at the Univ. of Wisconsin Hancock

Agricultural Research Station in Hancock. Chemical analysis showed that the soil had high P and K fertility as determined by extraction with  $\text{NH}_4\text{F-HCl}$  (Doll and Lucas, 1973; Thomas and Peaslee, 1973) and that the level of 1 mol·L<sup>-1</sup>  $\text{NH}_4\text{OAc}$ -extractable  $\text{Ca}^{2+}$  in the soil was 650 mg·kg<sup>-1</sup>, primarily as exchangeable  $\text{Ca}^{2+}$ .

A randomized complete-block design, with a factorial set of treatments, including 12 snap bean cultivars and four levels of gypsum (0, 1, 2, and 4 t·ha<sup>-1</sup>), with four replications, was used. Each plot was one row, 91 cm long, spaced 91 cm apart with 10 seeds per linear meter. All plots were fertilized with N at 40 kg·ha<sup>-1</sup> as  $\text{NH}_4\text{NO}_3$ , without supplemental P or K. Agricultural-grade gypsum was surface-applied when the plants had just emerged and the unifoliates were not expanded. The gypsum was spread by hand in a 15-cm band on each side of the row and was raked into the soil to a depth of 8 cm. Plots were irrigated bi-weekly as necessary to bring total rainfall + irrigation amounts to >25 mm·week<sup>-1</sup>.

For nine of the cultivars, 10 to 20 sieve size number 4 pods (a premium grade, 8.3 to 9.5 mm in diameter; Mullins and Straw, 1988) were hand-harvested on 27 Aug. 1994 from each plot. 'Astrel' and 'Slingreen' produce few or no size 4 beans and are considered at a premium grade when at sieve size 3 (7.3 to 8.3 mm in diameter) and, accordingly, were harvested at this stage. For the flat romano bean, 'Roma II', the harvested material represented a stage of physiological development similar to that of the other cultivars at harvest.

Harvested pods were oven-dried at 65 °C and ground in a Udy cyclone mill to pass through a 1-mm mesh sieve screen. Samples weighing 0.15 g were dry-ashed in a muffle furnace at 450 °C. Calcium in the ash was extracted using 2 mol·L<sup>-1</sup> HCl, filtered through Whatman no. 540 paper and adjusted to 1% La (w/v) for suppression of interferences. Calcium in the extracts was determined with a Varian atomic absorption spectrometer (Spectra AA-20; Mulgrave Victoria, Australia) and expressed on a dry mass (DM) basis. SAS system software (SAS Institute, 1992) was used to analyze the data. Differences between treatment means were separated using an LSD test (Steel and Torrie, 1980).

After harvest, soil samples were taken to a depth of 15 cm and composited to represent each level of gypsum applied. Saturated pastes (21.7% w/w) were prepared with sieved (<2 mm) soil samples and saturation extracts were prepared by vacuum filtration (Rhoades, 1982). These extracts were analyzed for  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{SO}_4^{2-}$ -S concentrations by simultaneous inductively coupled plasma-atomic emission spectroscopy (model 3400B; Applied Res. Lab., Valencia, Calif.) and electrical conductivity (EC) by a temperature compensating conductivity meter (Yellow Springs Instrument Co., Yellow Springs, Ohio). Saturation extracts were tested for gypsum saturation by chemical equilibria calculations using SPECIES (Barak, 1990). Data for the saturation extracts was recast for soil solutions at

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<sup>1</sup>To whom reprint requests should be addressed.

field moisture capacity (8.0% w/w) by first multiplying ion concentrations in the extract by a factor reflecting the difference in water content (2.7 = 21.7%/8.0%) and then iteratively calculating: 1) removal of Ca<sup>2+</sup> and SO<sub>4</sub><sup>2-</sup> from solution as gypsum precipitate, and 2) mole-for-mole cation displacement of exchangeable Ca<sup>2+</sup> into solution by Mg<sup>2+</sup> in order to achieve the same linear relationship between soluble Ca<sup>2+</sup> and Mg<sup>2+</sup> concentrations shown by the extract solutions. EC of soil solutions at field moisture capacity was calculated using the statistical relationship of Griffin and Jurinak (1973) based on calculated ionic strength.

### Results and Discussion

Even with the addition gypsum at rates as high as 4 t·ha<sup>-1</sup>, analysis of variance (ANOVA) revealed no significant effect of gypsum applied to the soil on the Ca<sup>2+</sup> concentration of snap bean pods (Table 1). In contrast, pod Ca<sup>2+</sup> concentrations differed highly significantly among the 12 snap bean cultivars evaluated in this study (Table 1, Fig. 1). Moreover, the differences among the snap bean cultivars were consistent over the levels of applied gypsum and no genotype × gypsum interaction was observed.

Soil analysis was used to evaluate the effect of the added gypsum on soil solution concentrations of Ca<sup>2+</sup>. Gypsum solubility is 2.63 g·L<sup>-1</sup> of water. The rates of gypsum application, 1, 2, and 4 t·ha<sup>-1</sup>, are sufficient to saturate soil solutions of Plainfield soil at the field moisture capacity (0.118 t·m<sup>-3</sup>) with respect to gypsum to depths of 32, 64, and 129 cm, respectively. Alternatively, since gypsum is a sparingly soluble salt applied as a surface application, the amounts added were sufficient to completely replenish the soil solution of the top 15 cm of field-moist Plainfield soil 2.1, 4.3, and 8.6 times, respectively, if applied gypsum was removed from the root zone by leaching due to irrigation or rainfall.

The ion activity product (Ca<sup>2+</sup>)(SO<sub>4</sub><sup>2-</sup>), where ( ) denotes activity, of the saturated water extract from the 4 t·ha<sup>-1</sup> gypsum treatment is 10<sup>-4.63</sup> (Table 2) and compares very closely with 10<sup>-4.64</sup> cited by Lindsay (1979) for gypsum. The extracts of the other gypsum applications were undersaturated. However, when saturation extract data were recast for soil solutions at field moisture capacity, these calculations demonstrated that soils that received gypsum at 2 or 4 t·ha<sup>-1</sup> were still gypsum-saturated at field moisture levels at the end of the growing season (Table 2). Both high gypsum application levels resulted in markedly higher concentrations of Ca<sup>2+</sup> in soil solution, i.e., they were about four times higher than for those receiving 0 and 1 t·ha<sup>-1</sup>.

Concentrations of Ca<sup>2+</sup> at field moisture capacity with 0 and 1 t·ha<sup>-1</sup> were higher than the median Ca<sup>2+</sup> concentrations of 24 nutrients solutions published by Jones (1983) and higher than the mode of 134 displaced soil solutions from Alfisols and Mollisols (Barber et al., 1962), indicating that the Ca<sup>2+</sup> concentrations of the soil solutions at field moisture capacity

Table 1. Analysis of variance for Ca concentration in the pods on 12 genotypes of snap beans, fertilized with four levels of gypsum.

Source	df	Mean square	F value	P > F
Block	3	4.07119	17.41	0.0001
Gypsum	3	0.47240	2.02	0.1147
Genotype	11	4.59784	19.66	0.0001
Gypsum × genotype	33	0.17594	0.75	0.8262
Error	141	0.23384		

CV = 9.59%

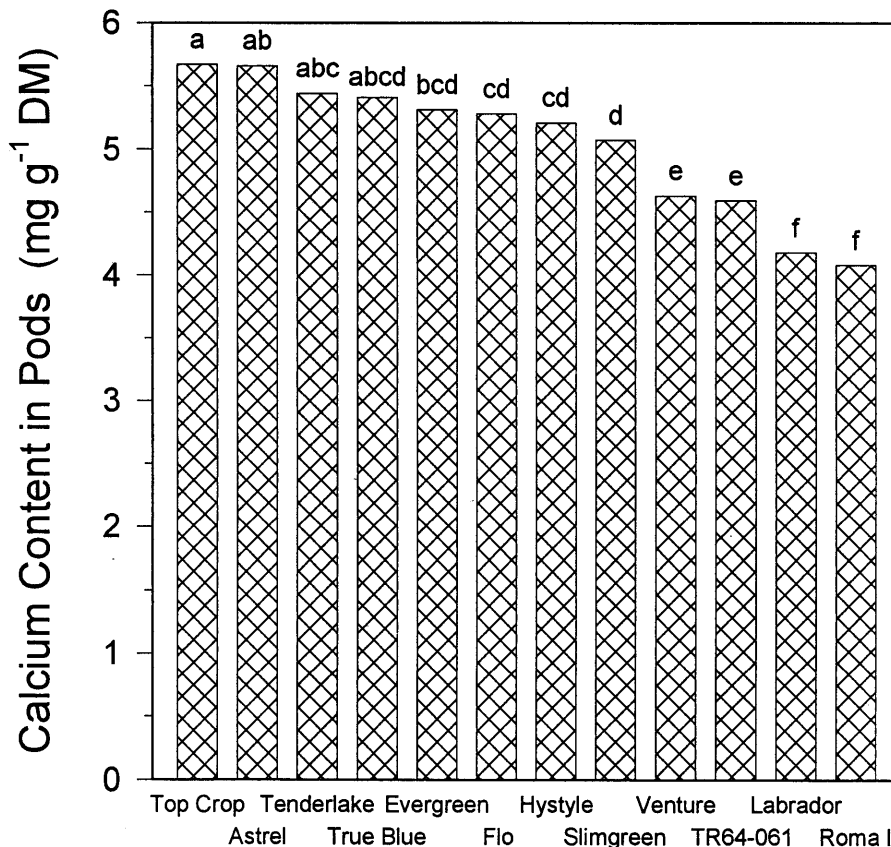


Fig. 1. Mean Ca concentration in pods of 12 snap bean cultivars. Means were separated by least significant differences at P ≤ 0.05.

Table 2. Selected soluble ions and electrical conductivity (EC) in saturated water extracts and calculated soil solution at field moisture capacity at four levels of gypsum applied to Plainfield loamy sand.

Gypsum applied (t·ha <sup>-1</sup> )	Saturation extract (measured)				Soil solution at field moisture capacity (calculated)			
	Ca <sup>2+</sup>	Mg <sup>2+</sup>	SO <sub>4</sub> <sup>2-</sup>	EC	Ca <sup>2+</sup>	Mg <sup>2+</sup>	SO <sub>4</sub> <sup>2-</sup>	EC
	(mmol·L <sup>-1</sup> )			(dS·m <sup>-1</sup> )	(mmol·L <sup>-1</sup> )			(dS·m <sup>-1</sup> )
0	1.21	0.81	0.32	0.56	4.2	1.3	0.9	1.41
1	1.16	0.57	0.78	0.49	3.5	1.2	2.1	1.23
2	6.08	1.74	7.14	1.51	15.1 <sup>z</sup>	3.4	16.6	4.02
4	14.97 <sup>z</sup>	3.37	16.81	2.50	16.1 <sup>z</sup>	3.6	15.6	4.25

<sup>z</sup>Saturated with respect to CaSO<sub>4</sub>·2H<sub>2</sub>O.

for the Plainfield loamy sand were at least sufficient for plant growth. In contrast, the EC of the soil saturation extracts of the 2- and 4-t·ha<sup>-1</sup> gypsum treatments approached the lower limit, 2 dS·m<sup>-1</sup>, at which yields of sensitive crops are restricted by salinity (U.S. Salinity Lab. Staff, 1954), indicating that yet higher levels of added soluble Ca<sup>2+</sup> might confound the increased Ca supply with growth reduction due to salinity. Results of this experiment, therefore, indicate that there is no significant increase in Ca<sup>2+</sup> concentration of snap bean pods when Ca<sup>2+</sup> in solution is at least suffi-

cient. Similarly, Brown et al. (1969) and Mix and Marschner (1976) did not obtain Ca<sup>2+</sup> dosage effects with snap bean fruit. Quite possibly, the plants are not responsive to changes in the level of available Ca<sup>2+</sup> in soil beyond sufficiency (Allaway, 1984).

The mean Ca<sup>2+</sup> concentration in pods of 12 cultivars averaged 5.0 mg·g<sup>-1</sup> DM and ranged from 4.1 to 5.7 mg·g<sup>-1</sup> DM (Fig. 1). 'Top Crop', 'Astrel', 'Tenderlake', and 'True Blue' were most able to concentrate Ca<sup>2+</sup> in their pods. A second group was composed of 'Evergreen', 'Flo', 'Hystyle', and 'Slimgreen'; 'Ven-

ture' and 'TR64-061' represent a third group. 'Labrador' and 'Roma II' had the lowest  $\text{Ca}^{2+}$  concentration in the pods. These results are in close agreement with Quintana et al. (1996) who tested a subset of these cultivars and reported pod  $\text{Ca}^{2+}$  concentrations of 6.55, 6.35, and 4.07  $\text{mg}\cdot\text{g}^{-1}$  for 'Hystyle', 'Evergreen', and 'Labrador', respectively. In the 1995 growing season, a partial replication of this experiment was conducted as a randomized complete-block design in triplicate at the same location using four cultivars ('Top Crop', 'Evergreen', 'Hystyle', and 'Labrador') and two levels of gypsum (0 and 200  $\text{kg}\cdot\text{ha}^{-1}$ ), with sampling and analysis as before. ANOVA for  $\text{Ca}^{2+}$  concentration in pods (data not shown) again showed a highly significant effect ( $P \leq 0.0001$ ) of genotype, but no significance to Ca addition as gypsum and no significant genotype  $\times$  Ca interaction.

The transport of  $\text{Ca}^{2+}$  to the snap bean pods is through the xylem (Mix and Marschner, 1976a). Since  $\text{Ca}^{2+}$  transport in the xylem is mainly a transpiration-driven process, the  $\text{Ca}^{2+}$  supply of plant parts with a low rate of transpiration, such as fruit, will be limited. Mix and Marschner (1976b) found a strong correlation between  $\text{Ca}^{2+}$  concentration in snap bean fruit and pod transpiration. However, simply increasing whole-plant transpiration does not necessarily increase the  $\text{Ca}^{2+}$  concentration of the fruit since leaves and fruit are in direct competition for both water and  $\text{Ca}^{2+}$  (Mix and Marschner, 1976b).

The causes of differences in  $\text{Ca}^{2+}$  concentration among cultivars in this experiment were not determined. Quintana et al. (1996) noted that pod size and  $\text{Ca}^{2+}$  concentrations are inversely correlated, which may be attributed to either a dilution factor in which a constant supply of  $\text{Ca}^{2+}$  is associated with varying amounts of dry matter or to differences in surface/volume relations affecting transpiration. Either cause may explain the inclusion of 'Astrel', with small pods, among those with high  $\text{Ca}^{2+}$  concentration in the pod, and 'Roma II', with a large and flat pod, as the cultivar with the lowest  $\text{Ca}^{2+}$  concentration. The pods of other cultivars were harvested at the same size, so the differences could not be attributed to this characteristic alone.

## Conclusion

Our results suggest that factors other than  $\text{Ca}^{2+}$  supply from the soil solution influenced the  $\text{Ca}^{2+}$  concentration of the snap bean pod. Enhanced  $\text{Ca}^{2+}$  concentration of pods may be better achieved through breeding and selection of cultivars with high  $\text{Ca}^{2+}$  concentration rather than adding  $\text{Ca}^{2+}$  amendments to the soil, when levels of  $\text{Ca}^{2+}$  in soil are otherwise sufficient.

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