Influence of Whole-plant Net Calcium Influx and Partitioning on Calcium Concentration in Snap Bean Pods

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Abstract. Snap beans (*Phaseolus vulgaris* L.) are a food source that can contribute to dietary Ca requirements in humans. Factors which might enhance the concentration of Ca in snap bean pods have been investigated by measuring whole-plant net Ca influx, whole-plant Ca partitioning, and various growth parameters in two snap bean cultivars—Hystyle and Labrador—that differ in pod Ca concentration. Plants were grown hydroponically under controlled environmental conditions while being provided adequate quantities of Ca. The concentration of Ca in pods (dry weight basis) was 52% higher in 'Hystyle', relative to 'Labrador', but net Ca influx throughout crop development or total plant Ca content at three stages of development were similar in both cultivars, demonstrating that pod Ca concentration differences were not due to differences in total plant Ca influx. However, 'Hystyle' partitioned more total plant Ca to pods, relative to 'Labrador'. Calcium flux analysis also revealed that daily rates of whole-plant net Ca influx gradually declined throughout the period of pod growth in both cultivars; this decline was not related to whole-plant water influx. These results suggest that enhancements in whole-plant net Ca influx during pod growth and/or enhancements in the xylem transport of absorbed Ca to developing pods could increase the Ca concentration of snap bean pods.

Calcium nutrition in humans is an important concern because of the structural role that Ca serves in bone development and the regulatory role that Ca plays in cellular metabolism (Linder, 1991). Although dairy products provide a major proportion of human dietary Ca needs (Chan et al., 1995), certain vegetable products can also provide significant levels of Ca, as well as other mineral nutrients, vitamins, and fiber [U.S. Dept. of Agriculture (USDA), 1984]. The absorption of Ca from foods, or its bioavailability, is dependent on factors in the gut as well as the occurrence of inhibitory components (e.g., phytate, oxalate) in the food source (Weaver and Heaney, 1991). Investigations involving humans have demonstrated that the bioavailability of Ca from vegetables which are low in phytates or oxalates (e.g., broccoli, bok choy, kale) is comparable to that of milk (about 50%; Heaney et al., 1993), while Ca bioavailability from spinach, which is high in oxalate, is only about 5% (Heaney et al., 1988). Inadequate Ca nutrition, especially during adolescent years, can lead to osteoporosis in later life (Johnston et al., 1992) and has been cited as a risk factor in colon cancer (Bruce, 1987); thus, the development of food sources with enhanced Ca nutritional quality could favorably affect human health.

Snap bean (*Phaseolus vulgaris*) is a vegetable that is high in Ca (USDA, 1984), low in inhibitory compounds (Vasquez Oderiz et al., 1994), and well liked by children (Pao et al., 1982), making it an ideal candidate for nutritional improvement. Investigations into the Ca nutrition of snap bean plants are limited, primarily focusing

on leaf concentrations of Ca in response to soil supplementation of other nutrients (Asif and Greig, 1972; Mack, 1983; Peck et al., 1989; Smith, 1977), rather than direct effects of fertilization on pod Ca concentrations (Peck and Van Buren, 1975). Early work demonstrated that transpiration and characteristics of the xylem pathway are important factors in the movement of Ca into and through the vegetative tissues of the bean plant (Bell and Biddulph, 1963; Biddulph et al., 1959, 1961); transpiration also has a primary role in the movement of Ca to the developing bean pod (Mix and Marschner, 1976a, 1976b). Only limited information exists on the long-term, whole-plant Ca requirements of the developing snap bean plant (Grusak et al., 1996).

Recently, a nearly 2-fold range of Ca concentrations in harvested pods was shown among 64 snap bean genotypes (Quintana et al., 1995); the physiological basis for these genetic differences has not been determined. Because we are interested in increasing the Ca concentration of snap bean pods, we have selected two genotypes from this previous study for further investigation: 'Labrador', which exhibited a low pod Ca concentration, and 'Hystyle', which exhibited an elevated pod Ca concentration (Quintana et al., 1995). Because only limited quantities of Ca are mobilized from older organs via the phloem stream (Raven, 1977), the transport of Ca to developing shoot organs depends both on net Ca influx via the roots and whole-plant partitioning of Ca via the xylem stream. Therefore, in both cultivars, daily rates of net Ca influx were measured throughout plant development, and whole-plant partitioning of Ca was determined at three time points during reproductive growth. Comparisons of the two cultivars will be used to discuss which physiological/biophysical factors may regulate the Ca nutrition of developing snap bean pods.

Materials and Methods

Plant material and growth. Two snap bean cultivars—Hystyle and Labrador—were grown in a recirculating hydroponic system (Grusak and Pezeshgi, 1994) situated within an environmental

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growth chamber (model PG2V; Controlled Environments Ltd, Winnipeg, Manitoba, Canada). Germination, planting, and growth of the plants were as previously described (Grusak et al., 1996). Basically, 60 plants were planted in the 10 growth containers of the hydroponic system; the total growing area was 3 m². Frames, built from plastic pipe, were positioned around plants for structural support. Nutrient solutions were prepared from reagent-grade chemicals and deionized water; the nutrient solution compositions (initial and refill solutions), which varied during development, have been previously described (Grusak et al., 1996). The recirculating solution was continuously aerated and pH was automatically controlled at pH 5.5 with KOH or H₂SO₄. Refill solutions were adjusted such that the concentrations of Ca²⁺ and Mg²⁺ were maintained in the recirculating hydroponic system within the ranges 0.44 to 0.12 mM and 0.50 to 0.24 mM, respectively (Grusak et al., 1996).

Environmental conditions for the plants were a temperature regime of 25 ± 0.5 °C, day and 20 ± 0.5 °C, night, with relative humidity held at 50% ± 5% throughout the day/night cycle. The photoperiod was maintained on a 14-h day, 10-h night; the 14-h day included 15 min at each end of the light period with the light intensity lowered to 50% of maximum. The light bank (containing both fluorescent and incandescent lamps) was positioned 1.2 m above the growth container lids and provided a PPF of 870 μmol·m⁻²·s⁻¹ at the top of the plants at final plant height (0.65 m).

Flux calculations. The recirculating hydroponic solution was periodically sampled (intervals of 2 or 3 days), refill solution usage was monitored, and the Ca concentration of hydroponic solution samples was determined using atomic absorption spectrophotometry (AAS) (model 2100; Perkin Elmer, Norwalk, Conn.). Net influx of Ca for individual plants (average of 60 plants) at each sampling interval was calculated from changes in the bulk hydroponic solution Ca concentration, along with the amounts of Ca entering the total system via the refill solution (Grusak and Pezeshgi, 1994; Grusak et al., 1996). Measurements of refill solution usage by the crop were used to determine daily rates of water uptake, also on an individual plant basis. Three crops (60

plants each) of both cultivars were grown for flux determinations.

Plant harvest and analysis. Additional crops were grown and plants were harvested on days 25, 35, and 50, which under our growth conditions corresponded to the beginning of developmental stages R6 (flowering), R7 (pod growth), and R8 (pod filling), respectively (Hall, 1991); plants of each cultivar were grown simultaneously in the recirculating hydroponic system. For the day 25 and day 35 harvests, two containers of six plants each were harvested for both cultivars; all plant tissues (leaves, stems, roots) were separated, but individual organ fractions were combined for the six plants in a given container. Data for these time points are thus the mean of two containers, with the value for each container being the mean of six plants. For the day 50 harvest, one container of six plants was harvested for each cultivar; leaves, stems, and pods were separated and collected on an individual plant basis. The roots, however, were combined due to the difficulty in separating roots of individual plants within the container. Throughout plant development, all senescing leaves were collected and combined with their respective plants of origin. Dry weights were determined for leaves, stems, and roots; fresh weights and dry weights were determined for pods. For dry weights, plant material was dried at 60 °C until a constant weight was attained. For fresh weights, pods were placed immediately in sealed plastic bags and refrigerated (4 °C) until weights could be determined. Pods were subsequently size graded according to United States standards for green beans (USDA, 1993) before drying.

Plant material was wet digested and the digestate resuspended as described in Grusak (1994). Total Ca content per gram dry weight of plant material was determined using AAS. Three subsamples of each organ fraction were analyzed and a mean value for the fraction was determined.

Plant tissue dry weights, Ca contents and Ca concentrations were subjected to an analysis of variance. Statistical significance of differences between mean values was determined using the Student *t* test.

Results

Fig. 1. Daily rates of net Ca influx determined for two snap bean cultivars, 'Hystyle' and 'Labrador'. Plants were grown at a density of 20 plants/m²; data points for each variety were determined from three separate crops of 60 plants. Reproductive growth stages (R6, R7, R8) are indicated at the top of the figure.

Flux characteristics. Daily rates of net Ca influx, calculated on a per-plant basis, increased through early growth until developmental stage R6 (days 25 to 35), and then continually declined throughout the remainder of the measurement period (Fig. 1). Peak rates for both cultivars were about 0.40 to 0.45 mmol·d⁻¹ per plant. The developmental changes in whole-plant net Ca influx were similar in the two cultivars (Fig. 1).

Whole-plant water influx was measured to compare its relationship to that of the Ca fluxes. Similar to net Ca influx, water influx increased throughout early vegetative growth and into developmental stage R6, but then maintained a roughly constant rate until the time of pod harvest, rather than exhibiting a decline (Fig. 2). The maximal rate for both cultivars was approximately 0.4 L·d⁻¹ per plant; flux changes during crop development were identical in the two cultivars.

Weight and Ca content of plant organs. Plant growth, as determined by dry weight measurements of plant organs at three time points, was comparable in the two cultivars. The only significant difference was in the stem fraction which was larger in 'Labrador' than in

'Hystyle' at day 25; the stem fractions were similar in mass at later time points. Almost all weight gain from day 35 to 50 was attributable to pod growth in plants of both cultivars. Average dry weight of fractions at day 50 (g/plant) for 'Hystyle' were 11.9, leaves; 8.6, stems; 2.5, roots; and for 'Labrador' were 12.3, leaves; 10.4, stems; 2.6, roots.

Age-related changes in Ca content were measured in leaves, stems, roots and total plant. Similar to the weight measurements, there were no significant differences between 'Hystyle' and 'Labrador' in Ca accretion for any organ except stems (day 25 only). Calcium content increased in leaves, roots, and in the total plant from day 25 to 50 in both cultivars. Calcium content in the stem fraction was unchanged after day 35 in 'Hystyle' and was maximal no later than day 25 in 'Labrador'. Average Ca content of fractions at day 50 (mg Ca/plant) for 'Hystyle' were 327.8, leaves; 55.4, stems; 9.6, roots; and for 'Labrador' were 395.2, leaves; 45.9, stems; 12.8, roots.

Of the pod characteristics measured (Table 1), the only significant differences noted were in pod Ca concentrations based either on fresh weight or dry weight. In addition, the percent of total plant Ca that was recovered in pods at the time of harvest also was significantly higher in 'Hystyle' (6.9%) than in 'Labrador' (5.2%).

Harvested pods were separated according to USDA grade sizes. The distribution of pods in each grade size, calculated as a percent of total harvested pod fresh weight, was identical in the two cultivars, being size 1, 2%; size 2, 12%, size 3, 23%; size 4, 58%, and size 5, 5%.

Discussion

Factors influencing pod Ca concentration. To understand the processes which contribute to the nutrition of developing snap bean pods, we have assessed net Ca influx via roots, Ca partitioning to all plant organs, and growth parameters in two cultivars which previously have been shown to differ in the Ca concentration of their harvested pods (Quintana et al., 1995). Plants for this study were grown hydroponically under controlled nutritional and environmental conditions, to ensure an adequate supply of Ca to the roots and to alleviate any biological or physical stress to the plants. Under our growth conditions, pod Ca concentration was 52% higher in 'Hystyle' relative to 'Labrador' (Table 1); general growth characteristics of the cultivars were identical, except for an earlier increase in mass of the stem fraction in 'Labrador'.

Total plant Ca content did not differ between 'Hystyle' and 'Labrador', nor did daily rates of net Ca influx (especially during pod growth; Fig. 1), demonstrating that the elevated Ca concentration of 'Hystyle' pods (Table 1) was not a result of enhanced net Ca influx at the whole-plant level. Thus, to explain the cultivar variation, these results pointed either to differences in total pod yield, or to differences in the partitioning of absorbed Ca into pods. Pod yield did not differ between cultivars on either a fresh or dry weight basis (Table 1), nor did the absolute Ca content of pods on an individual plant basis (Table 1). However, because there was a tendency towards more total Ca in 'Hystyle' pods, and because of the variability we had observed in whole-plant Ca content and weight, we also calculated the partitioning of Ca to pods as a

percent of total plant Ca (Table 1). Our results confirmed that Ca concentrations were elevated in 'Hystyle' pods, relative to 'Labrador' pods, because a higher percentage of the absorbed Ca was delivered to pods in 'Hystyle' plants.

Because Ca is poorly mobilized in the phloem, its transport to developing pods will depend upon the biophysics of transit through the xylem pathway and the factors that regulate transpiration-driven flow (Clarkson, 1984). Mix and Marschner (1976b) demonstrated that pod transpiration was responsible for Ca transport into developing bean pods; pod Ca import could be inhibited by increasing the relative humidity of the air space surrounding the pods. However, ionic exchange sites along the conducting pathway also have been shown to affect the movement and free activity of Ca in bean stems (Bell and Biddulph, 1963). In addition, the cell wall properties and solute concentration of growing tissues can influence growth-induced water influx (Cosgrove, 1986; Nonami and Boyer, 1987), a process supplemental to transpiration-driven flow. Further studies are needed to determine which of these physiological/biophysical factors contribute to the observed Ca differences in 'Hystyle' and 'Labrador' pods. It is interesting to note, however, that the pod Ca concentrations observed in this study under growth chamber conditions (Table 1) were lower in both cultivars than those found under field conditions (Quintana, Harrison, Nienhuis, Palta and Grusak, unpublished results); this is due perhaps to the denser canopy in hydroponic growth chamber-grown plants that results in a more humid microenvironment around the pods.

Developmental changes in net Ca influx. Both snap bean cultivars exhibited distinct developmental changes in daily rates of net Ca influx, which gradually declined throughout the period of pod growth (Fig. 1). The reason for this decline is unknown, but it should be recognized that it is not related to the root influx of water; whole-plant water influx was relatively unchanged in both 'Hystyle' and 'Labrador' during the period of declining net Ca influx (Figs. 1 and 2).

One consequence of the decline in net Ca influx (with no change in water influx) is that during the period of pod development (i.e.,

Fig. 2. Daily rates of whole-plant water influx determined for two snap bean cultivars, 'Hystyle' and 'Labrador'. Plants were grown at a density of 20 plants/m²; data points for each variety were determined from three separate crops of 60 plants. Reproductive growth stages (R6, R7, R8) are indicated at the top of the figure.

Table 1. Calcium content and weight characteristics of snap bean pods at harvest. Plants were grown hydroponically at a density of 20 plants/m².

Cultivar	Total Ca ^{zy} (mg)	Fresh wt (g)	Dry wt (g)	Ca (mg)/ g fresh wt	Ca (mg)/ g dry wt	Percent of total plant Ca
Hystyle	29.7 a	119.7 a	7.8 a	0.25 a	3.8 a	6.9 a
Labrador	22.7 a	132.2 a	9.5 a	0.19 b	2.5 b	5.2 b

^zPod Ca content and weight were determined on an individual plant basis; values represent means of six plants.

^yValues within a column followed by different letters are significantly different (*t* test, *P* < 0.01).

day 35 to 50), the concentration of Ca in the xylem sap also will be declining. Thus, as a pod matures, the molar quantity of Ca will be less in a given volume of xylem sap on each succeeding day. This may explain, in part, the observation that pod Ca concentration declines with increasing pod maturity (i.e., increasing grade sizes) (Mills and Jones, 1979; Mix and Marschner, 1976b); the daily ratio of Ca import (from xylem sap) to total dry matter import (predominantly from phloem sap) appears to diminish with pod maturation. In our study, no differences in grade size distribution were observed between harvested pods of 'Hystyle' or 'Labrador' (see Results), and thus cannot account for the genotypic variation in pod Ca concentration.

Potential for enhancing pod Ca concentration. Our Ca partitioning results have reinforced earlier observations that xylem-mediated transport is a primary determinant of Ca nutrition in snap bean pods. Thus, modification of any factor that would enhance pod transpiration (e.g., more open canopy structure, increased pod stomatal conductance) should help to increase the total import of Ca into pods. If pod transpiration were increased, it would not be expected to negatively impact total Ca nutrition of the plant. Pods would merely absorb a greater percentage of recently absorbed Ca; leaf function presumably would be unaffected due to sufficient Ca from prior acquisition. Alternatively, if whole-plant net Ca influx could be maintained at its maximal rate (i.e., day 35, Fig. 1) throughout the period of pod growth, a consistent Ca concentration in the xylem sap also would serve to enhance the Ca concentration of pods. Besides the obvious benefit that this enhancement would have in providing more dietary Ca to humans who consume snap beans, it also should be noted that the Ca content of bean seeds (Mix and Marschner, 1976c) and textural properties of processed snap beans (Stanley et al., 1995) would benefit, as well.

Literature Cited

- Asif, M.I. and J.K. Greig. 1972. Effects of seasonal interactions of nitrogen, phosphorus, and potassium fertilizers on yield and nutrient content of snap beans (*Phaseolus vulgaris* L.). J. Amer. Soc. Hort. Sci. 97:44–47.
- Bell, C.W. and O. Biddulph. 1963. Translocation of calcium. Exchange versus mass flow. Plant Physiol. 38:610–614.
- Biddulph, O., R. Cory, and S. Biddulph. 1959. Translocation of calcium in the bean plant. Plant Physiol. 34:512–519.
- Biddulph, O., F.S. Nakayama, and R. Cory. 1961. Transpiration stream and ascension of calcium. Plant Physiol. 36:429–436.
- Bruce, W.R. 1987. Recent hypotheses for the origin of colon cancer. Cancer Res. 47:4237–4242.
- Chan, G.M., K. Hoffman, and M. McMurry. 1995. The effects of dairy products on bone and body composition in pubertal girls. J. Pediatr. 126:551–556.
- Clarkson, D.T. 1984. Calcium transport between tissues and its distribution in the plant. Plant Cell Envir. 7:449–456.
- Cosgrove, D.J. 1986. Biophysical control of plant cell growth. Annu. Rev. Plant Physiol. 37:377–405.
- Grusak, M.A. 1994. Iron transport to developing ovules of *Pisum sativum*. I. Seed import characteristics and phloem iron-loading capacity of source regions. Plant Physiol. 104:649–655.
- Grusak, M.A. and S. Pezeshgi. 1994. Uniformly ¹⁵N-labeled soybean seeds produced for use in human and animal nutrition studies: Description of a recirculating hydroponic growth system and whole plant nutrient and environmental requirements. J. Sci. Food Agr. 64:223–230.
- Grusak, M.A., S. Pezeshgi, K.O. O'Brien, and S.A. Abrams. 1996. Intrinsic ⁴²Ca-labelling of green bean pods for use in human bioavailability studies. J. Sci. Food Agr. 70:11–15.
- Hall, R. 1991. Compendium of bean diseases. Amer. Phytopathol. Soc. Press, St. Paul, Minn.
- Heaney, R.P., C.M. Weaver, S.M. Hinders, B. Martin, and P.T. Packard. 1993. Absorbability of calcium from *Brassica* vegetables: broccoli, bok choy, and kale. J. Food Sci. 58:1378–1380.
- Heaney, R.P., C.M. Weaver, and R.R. Recker. 1988. Calcium absorbability from spinach. Amer. J. Clin. Nutr. 47:707–709.
- Johnston, Jr, C.C., J.Z. Miller, C.W. Slemenda, T.K. Reister, S. Hui, J.C. Christian, and M. Peacock. 1992. Calcium supplementation and increases in bone mineral density in children. New England J. Med. 327:82–87.
- Linder, M.C. 1991. Nutritional biochemistry and metabolism: With clinical applications. 2nd ed. Elsevier, New York.
- Mack, H.J. 1983. Fertilizer and plant density effects on yield performance and leaf nutrient concentration of bush snap beans. J. Amer. Soc. Hort. Sci. 108:574–578.
- Mills, H.A. and J.B. Jones, Jr. 1979. Elemental content of frozen snap bean fruit. HortScience 14:268–269.
- Mix, G.P. and H. Marschner. 1976a. Calciumgehalte in Früchten von Paprika, Bohne, Quitte und Hagebutte im Verlauf des Fruchtwachstums (Calcium content in fruits of paprika, bean, quince and hip during fruit growth). Z. Pflanzenernaehr. 5:537–549.
- Mix, G.P. and H. Marschner. 1976b. Calcium-Umlagerung in Bohnenfrüchten während des Samenwachstums (Redistribution of calcium in bean fruits during seed development). Z. Pflanzenphysiol. 80:354–366.
- Mix, G.P. and H. Marschner. 1976c. Einfluß exogener und endogener Faktoren auf den Calciumgehalt von Paprika- und Bohnenfrüchten (Effect of external and internal factors on the calcium content of paprika [pepper] and bean fruits). Z. Pflanzenernaehr. 5:551–563.
- Nonami, H. and J.S. Boyer. 1987. Origin of growth-induced water potential. Solute concentration is low in apoplast of enlarging tissues. Plant Physiol. 83:596–601.
- Pao, E.M., K.H. Fleming, P.M. Guenther, and S.J. Mickle. 1982. Foods commonly eaten by individuals: amount per day and per eating occasion. Consumer Nutr. Center, Human Nutr. Info. Serv., U.S. Dept. of Agr., Home Econ. Res. Rpt. 44.
- Peck, N.H., G.E. MacDonald, and A.V. Gardner. 1989. Snap bean plant responses to sources and rates of nitrogen and potassium fertilizers. HortScience 24:619–624.
- Peck, N.H. and Van Buren, J.P. 1975. Plant response to concentrated superphosphate and potassium chloride fertilizers. V. Snap bean (*Phaseolus vulgaris* var. *humilis*). N.Y. State Agr. Expt. Sta., Geneva. SEARCH 5(2):1–32.
- Quintana, J.M., H.C. Harrison, and J. Nienhuis. 1995. Variation for calcium concentration among 64 genotypes of snap bean (*Phaseolus vulgaris*). HortScience 30:797. (Abstr.)
- Raven, J.A. 1977. H⁺ and Ca²⁺ in phloem and symplast: relation of relative immobility of the ions to the cytoplasmic nature of the transport paths. New Phytol. 79:465–480.
- Smith, C.B. 1977. Growth responses, nutrient leaf concentrations and interelement relationships of snap beans as affected by fertilizer treatment. J. Amer. Soc. Hort. Sci. 102:61–64.
- Stanley, D.W., M.C. Bourne, A.P. Stone, and W.V. Wismer. 1995. Low temperature blanching effects on chemistry, firmness and structure of canned green beans and carrots. J. Food Sci. 60:327–333.
- U.S. Department of Agriculture. 1984. USDA agricultural handbook series 8. Composition of foods: Vegetables and vegetable products—Raw, processed, prepared. Nutrition Monitoring Div., Human Nutrition Info. Serv., U.S. Dept. of Agr., Hyattsville, Md.
- U.S. Department of Agriculture. 1993. United States standards for grades of canned green beans and canned wax beans. Fed. Reg. 58:4295–4302.
- Vazquez Oderiz, M.L., M.E. Vazquez Blanco, J. Lopez Hernandez, J. Simal Lozano, and M.A. Romero Rodriguez. 1994. Simultaneous determination of organic acids and vitamin C in green beans by liquid chromatography. J. AOAC Intl. 77:1056–1059.
- Weaver, C.M. and R.P. Heaney. 1991. Isotopic exchange of ingested calcium between labeled sources. Evidence that ingested calcium does not form a common absorptive pool. Calcif. Tissue Intl. 49:244–247.