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Differences in Pod Calcium Concentration for Eight Snap Bean and Dry Bean Cultivars

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Abstract. **This study was designed to compare snap and dry beans (*Phaseolus vulgaris* L.) for pod Ca concentration, and to identify genetic resources that might be useful in breeding programs directed to increase Ca concentration in bean pods. Pods from eight snap bean and eight dry bean cultivars were evaluated for Ca concentration during 1995 and 1996 at Hancock, Wis. A randomized complete-block design was utilized with three replications in 1995 and six in 1996. Beans were planted in June and hand-harvested in August for both experiments. Soil Ca at planting time was 580 mg·kg^{-1} in 1995 and 500 mg·kg^{-1} in 1996. No additional Ca was added. Plots consisted of 10 plants each. At harvest, a pooled sample of 10 to 15 size no. 4 pods was collected from each plot. Atomic absorption spectrophotometry was used to determine Ca content. Significant differences ($P \leq 0.01$) were detected among and within bean types (dry and snap). Although bean type × year interaction was nonsignificant, a strong year effect was observed ($P \leq 0.01$). Snap beans (4.6 ± 0.7 mg·g^{-1} dry weight) had significantly higher pod Ca concentration than did dry beans (4.2 ± 0.6 mg·g^{-1} dry weight). Within snap beans, 'Checkmate' had the highest pod Ca concentration (5.5 ± 0.3 mg·g^{-1} dry weight) and 'Nelson' the lowest (3.8 ± 0.3 mg·g^{-1} dry weight). Within dry beans, 'GO122' had the highest (5.1 ± 0.4 mg·g^{-1} dry weight) and 'Porrillo 70' the lowest pod Ca concentration (3.6 ± 0.3 mg·g^{-1} dry weight). Six cultivars had pod Ca concentrations significantly ($P \leq 0.01$) higher than the overall mean (4.4 ± 0.3 mg·g^{-1} dry weight).**

The economic importance and popularity of beans have been recognized since ancient times (Rubatzky and Yamaguchi, 1997). Beans are mainly eaten in two forms, as dry beans (i.e., seeds) and as snap beans (i.e., immature pods). Use and consumption of beans vary with geographical area (Food and Agriculture Organization, 1990). People in developing countries usually eat more dry beans than do those in more industrialized countries. Beans represent a good and inexpensive source of protein, vitamins, and minerals (Drummond, 1996). Previous studies have shown beans to be a good Ca source among vegetables (Stevens, 1974). Dry beans (seeds) contain ≈1.6 mg·g^{-1} dry weight Ca (Peirce, 1987), while snap beans (pods) average 5.6 mg·g^{-1} dry weight (Ensminger et al., 1994). Due to relatively low quantities of Ca inhibitors, such as oxalic acid (U.S. Dept. of Agriculture, 1984), Ca contained in snap beans is readily adsorbed by humans (Grusak et al., 1996). A 100-g (1 cup) serving of uncooked snap beans can provide 56 mg of Ca (Ensminger et al., 1994), or ≈5% of the minimum daily Ca requirement of 1200 mg (U.S. National Research Council, 1989). Thus, inclusion of snap beans in the diet may help prevent diseases related to a lack of Ca, such as osteoporosis (Herbert et al., 1990).

Genetic diversity within and among plant species for nutrient uptake, distribution, and use has been recognized for many years (Clark and Brown, 1980; Epstein and Jefferies, 1964; Vose, 1963). Mechanisms by which specific plants grow under mineral stress differ among both species and minerals (Clark, 1983).

In snap beans, genetic variability has been detected for acquisition and use of K (Shea et al., 1967), P (Fawole et al., 1982), and Ca (Quintana et al., 1996). The physiological bases for variability in pod Ca concentration among snap bean cultivars are not well understood; however, recent studies indicate that differences in root pressure could be important (Quintana, 1998). Attempts to increase pod Ca concentration in snap beans through fertilization with several types (Quintana, 1998) and rates (Miglioranza et al., 1997) of commercial Ca sources have failed, suggesting that increasing pod Ca concentration in beans may be achieved better through breeding.

Knowledge concerning the variability in Ca concentration in snap bean pods is still limited. In addition to the more conventional goals of improved yield and disease and insect resistances, breeding programs are beginning to focus on improving the nutritional quality of vegetables. To increase pod Ca concentration more efficiently in beans, scientists need insights into the variability that currently exists in order to use the best genotypes in their breeding programs. Thus, the purpose of this study was to explore the variability in pod Ca concentration in beans. It was designed to compare snap and dry beans, as well as to identify new resources that could be useful in breeding studies.

Materials and Methods

Plant material. Sixteen bean cultivars commonly used in breeding programs worldwide were evaluated in this study. Eight snap bean and eight dry bean cultivars were selected, based on their geographical origin, phenotypic differences, and market classes, to include a range of variability for color and shape of seed and pod, use (fresh or processing), disease tolerance (mainly to soil pathogen complexes), and yield. Snap bean cultivars were 'Astro', 'Checkmate', 'Evergreen', 'Hystyle', 'Labrador', 'Nelson', 'TR67.042.211', and 'Unidor'. All but 'Unidor' (wax bean) had green pods, and all originated in the United States except 'Unidor' and 'Evergreen', which were developed in Europe. Dry beans utilized were 'A55', 'BM3.056', 'Carioca', 'GN1140', 'GO122', 'K407', 'Porrillo', and 'Puebla 152'. Their geographical areas of use and origin included Brazil ('Carioca'), Mexico ('Puebla 152'), and India ('GO122'). Color, size, and shape of seed differed among these cultivars, which included black seeded ('A55', 'Puebla 152', and 'Porrillo'), kidney shaped ('K407' and 'GO122'), large white seeded or Great Northern ('GN1140'), Navy ('BM3.056'), and brown-beige seeded ('Carioca') types.

Experimental design and analysis. The field experiments were conducted at the Univ. of Wisconsin–Research Station in Hancock. All bean cultivars were planted in a randomized complete-block design (Fryer, 1966), replicated three times in 1995 and six times in 1996. The soil was sandy loam having 1.2% organic matter. The pH was 6.1 in 1995 and 6.4 in 1996. Soil concentrations of P_2O_5, K_2O, and Ca were 88, 107, and 580 mg·kg^{-1} in 1995 and 72, 75, and 500 mg·kg^{-1} in 1996. No additional Ca was added to the soil either year. Analysis of variance was performed on the data using SAS system software (SAS Institute, Cary, N.C.), and cultivar means were separated by least significant difference (Fryer, 1966).

Plant culture and sampling. Beans were planted on 5 June 1995 and 20 June 1996, with each plot consisting of one 1.02-m row. In each row, 20 seeds were double seeded, and seedings were thinned ≈2 weeks after planting

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to 10 per row, 10.2 cm apart. Blocks and rows were spaced 91 cm apart with guard rows planted along the periphery of the experiments, and the total area of each plot was ≈0.93 m^2.

Standard cultural practices were followed including: preplant incorporation of the herbicide trifluralin (α,α,α,-trifluoro-2,6-dimitro-*N*,*N*-dipropyl-p-toluidine), applications of acephate (methamidophos *O*,*S*-dimethyl phosphoramidothioate) insecticide as needed for leafhoppers, cultivation (20 to 30 d after planting) for weed control, one sidedressed fertilizer application (33.5N–0P–0K) at 100 $kg \cdot ha^{-1}$ ≈2 weeks after planting, and 12.5 mm of irrigation water per week from planting time to harvest (Binning et al., 1999). Beans flowered 40 to 45 d after planting. However, considerable variability was observed, with dry beans flowering several days later than snap beans. Most pods were full and seeds small (pod filling) at harvest (Hall, 1991).

Even though dry beans are used mainly as mature seeds, pods were collected from both dry and snap beans at the same physiological age in order to compare pod Ca concentration. Criteria for sampling were based on seed size, using a commercial pocket pod grader to check for maturity. Plots were monitored every 2 d starting 2 weeks after flowering (≈55 to 60 d after planting). To ensure proper comparison for pod Ca concentration among cultivars, pods were harvested when most of the seeds were 5 mm long, corresponding to commercial sieve size number 4 (Peck et al., 1989). A pooled sample of 10 to 15 pods was randomly taken from the 10 plants in each plot for Ca determinations.

Laboratory analysis. After harvest, pods were oven-dried at 60 to 65 °C for 48 h, then ground in a Wiley mill to pass a 10-mesh screen. A 0.05-g sample for each treatment was weighed and placed in a 10-mL glass beaker. Samples were dry ashed in a muffle furnace at 450 °C for 5 h. After cooling, the Ca was extracted by adding 5 mL of 2 N HCl. This solution was poured through Whatman no. 540 filter paper and collected in a 50-mL volumetric flask. The filter paper was rinsed with two to three volumes of distilled-deionized water to ensure that all Ca was extracted from the ash. Finally, 10 mL of 0.2 N HCl containing 10 $g \cdot L^{-1}$ lanthanum (as $LaCl_3$) was added to the Ca extract to overcome chemical interferences, and total volume was brought to 50 mL with distilled-deionized water (Greweling, 1976). Calcium concentrations were determined with an atomic absorption spectrophotometer (model SpectrAA-20; Varian Techtron Pty. Ltd., Mulgrave Victoria, Australia). Standards used for calibration were: 0, 1.0, 2.0, 3.0, 4.0, and 5.0 $mg \cdot L^{-1}$ of Ca. When a sample had higher Ca concentration than the highest standard, it was diluted 50% with a solution of 0.2 N HCl containing 2000 $mg \cdot L^{-1}$ lanthanum (as $LaCl_3$) (Varian, 1986).

Results and Discussion

Overall mean Ca concentration in dried pod tissue for all cultivars evaluated was 4.4 ± 0.3 $mg \cdot g^{-1}$, which was similar to the overall mean of 4.8 ± 0.9 $mg \cdot g^{-1}$ found in previous studies (Quintana et al., 1996). Two dry beans, 'GO122' (5.1 $mg \cdot g^{-1}$) and 'Carioca' (4.9 $mg \cdot g^{-1}$), and four snap beans, 'Checkmate' (5.5 $mg \cdot g^{-1}$), 'Hystyle' (5.2 $mg \cdot g^{-1}$), 'Evergreen' (5.0 $mg \cdot g^{-1}$), and 'TR67.042.211' (4.9 $mg \cdot g^{-1}$) had pod Ca concentrations significantly higher than the overall mean (Table 1). The mean pod Ca concentration (across years and replications) was 8.7% higher for snap beans (4.7 ± 0.7 $mg \cdot g^{-1}$) than for dry beans (4.2 ± 0.6 $mg \cdot g^{-1}$), significant at $P \leq 0.001$. Mean pod Ca concentration (tissue dry weight) for all cultivars ranged from a high of 5.5 ± 0.3 $mg \cdot g^{-1}$ for 'Checkmate' to a low of 3.6 ± 0.3 $mg \cdot g^{-1}$ for 'Porrillo 70'. Pod Ca concentration differed significantly among snap bean cultivars. 'Checkmate' had the highest concentration and 'Nelson' the lowest (3.8 ± 0.3 $mg \cdot g^{-1}$), supporting previous work on snap beans (Quintana et al., 1996). Within dry beans the highest value was 5.1 ± 0.4 $mg \cdot g^{-1}$ for 'GO122' and the lowest (3.6 ± 0.3 $mg \cdot g^{-1}$) for 'Porrillo 70'. Thus, variability for pod Ca concentration in dry beans was as high as that found in snap beans. In general, Ca concentration in dry bean cultivars was 162% higher in immature pods (4.2 ± 0.6 $mg \cdot g^{-1}$) than values reported for mature seeds (1.6 $mg \cdot g^{-1}$) in other studies (Peirce, 1987). Bean cultivars ranked as high accumulators of pod Ca might be used in genetic studies designed to establish the genetic bases for such accumulation.

Environmental effects. No type × year interaction was observed ($P = 0.109$), suggesting that snap beans had higher pod Ca concentrations than dry beans regardless of year. Similarly, cultivar (type) × year interaction was nonsignificant ($P = 0.429$), which agrees with previous studies (Quintana, 1998; Quintana et al., 1996). Bean breeding for pod Ca concentration may be facilitated by these findings, because consistency of the rankings across environments suggests that evaluation and selection could be effective in one location.

Significant differences were found between years for pod Ca concentration ($P \leq 0.001$), indicating an environmental influence. Mean pod Ca concentration was 7% higher in 1995 than in 1996 (Table 1). Because soil Ca levels in 1995 (580 $mg \cdot kg^{-1}$) were 14% higher than in 1996 (500 $mg \cdot kg^{-1}$), differences among years for pod Ca concentration might be attributed to differences in soil Ca content. However, recent studies have shown than an increase in soil Ca beyond sufficiency does not increase snap bean pod Ca concentrations (Miglioranza et al., 1997; Quintana, 1998).

Differences among years might be explained by temperature and rainfall variation. Total accumulated heat units (base 10 °C) for the 1995 growing season (1734) were 23% higher than those in the 1996 season (1339). Similarly, rainfall during the 1995 growing season (318 mm) was 43% higher than that during the 1996 season (183 mm). Accumulated heat units and rainfall are important for Ca accumulation in pods, and root pressure is an important mechanism for Ca accumulation in pods (Quintana, 1998). Lower temperatures and soil moisture can significantly reduce root pressure (Kramer, 1983). Thus, the lower pod Ca accumulation in 1996 may have reflected the influence of environment on root pressure.

Table 1. Effects of cultivar, type and year on pod calcium concentration ($mg \cdot g^{-1}$ dry weight) for eight snap bean and eight dry bean cultivars grown at one location over 2 years.

Factor[z]	Bean type	Mean ± SE
Year		
1995	All	4.6 ± 0.7 a[y]
1996	All	4.3 ± 0.6 b
Type		
Both years	Snap	4.6 ± 0.7 a
Both years	Dry	4.2 ± 0.6 b
Cultivar		
Checkmate	Snap	5.5 ± 0.3 a
Hystyle	Snap	5.2 ± 0.2 ab
GO122	Dry	5.1 ± 0.4 b
Evergreen	Snap	5.0 ± 0.3 b
Carioca	Dry	4.9 ± 0.3 bc
TR67.042.211	Snap	4.8 ± 0.3 b–d
Astro	Snap	4.6 ± 0.4 c–e
GN1140	Dry	4.5 ± 0.3 de
K407	Dry	4.4 ± 0.3 ef
Puebla 152	Dry	4.1 ± 0.4 fg
Unidor	Snap	3.9 ± 0.4 gh
Labrador	Snap	3.9 ± 0.2 gh
BM3.056	Dry	3.8 ± 0.3 gh
Nelson	Snap	3.8 ± 0.3 gh
A55	Dry	3.7 ± 0.2 h
Porrillo 70	Dry	3.6 ± 0.3 h
Overall mean		4.4 ± 0.3
CV	6.7%	

[z]No interactions were significant at $P \leq 0.05$.
[y]Mean separation within factors.

This research demonstrates that variation for pod Ca concentration exists among snap and dry beans and that dry beans accumulate less Ca in their pods than do snap beans. The nonsignificant type × year or cultivar × year interactions suggest that environmental variables may not be significant factors in selection, despite differences in accumulated heat units (base 10 °C) and rainfall. Of the 16 genotypes evaluated, the six with highest pod Ca concentrations may be useful in breeding programs targeted to enhance this characteristic.

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