Comparison of Pod Calcium Concentration between Two Snap Bean Populations

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ADDITIONAL INDEX WORDS. heritability, genetic variance, bean breeding, plant nutrition

ABSTRACT. To understand the genetics that control pod Ca concentration in snap beans, two snap bean (*Phaseolus vulgaris* L.) populations consisting of 60 genotypes, plus 4 commercial cultivars used as checks, were evaluated during Summers 1995 and 1996 at Hancock, Wis. These populations were CA2 ('Evergreen' x 'Top Crop') and CA3 ('Evergreen' x 'Slimgreen'). The experimental design was an 8×8 double lattice repeated each year. No Ca was added to the plants grown in a sandy loam soil with 1% organic matter and an average of 540 ppm Ca. To ensure proper comparison for pod Ca concentration among cultivars, only commercial sieve size no. 4 pods (a premium grade, 8.3 to 9.5 mm in diameter) were sampled and used for Ca extractions. After Ca was extracted, readings for Ca concentration were done via atomic absorption spectrophotometry. In both populations, genotypes and years differed for pod Ca concentration ($P = 0.001$). Several snap bean genotypes showed pod Ca concentrations higher than the best of the checks. Overall mean pod Ca concentration ranged from a low of 3.82 to a high of 6.80 mg·g^{-1} dry weight. No differences were detected between the populations. Significant year × genotype interaction was observed in CA2 ($P = 0.1$), but was not present in CA3. Population variances proved to be homogeneous. Heritability for pod Ca concentration ranged from 0.48 (CA2) to 0.50 (CA3). Evidently enhancement of pod Ca concentration in beans can successfully be accomplished through plant breeding.

Beans are a major source of protein in the Americas and parts of Asia and Africa where animal products are scarce or too expensive for widespread consumption (Bliss and Brown, 1983). Snap beans also have high Ca levels (Grusak et al., 1996) as well as being a popular vegetable ranking fourth (canned), sixth (frozen), and fourteenth (fresh) for per capita consumption among major vegetables (National Agricultural Statistics Service, 1997), indicating that they have the potential to contribute a significant amount of Ca to the human diet. In addition, Ca contained in snap beans is readily adsorbed by humans (Grusak et al., 1996). Thus any increase of Ca accumulation in bean pods, could impact human nutrition, especially in developing countries, societies having Ca intake deficits, and individuals suffering from Ca deficiency related diseases such as osteoporosis (Bronner, 1994).

Differences among and within species for plant requirements of essential mineral elements have been recognized since early times (Harvey, 1939). In common beans (*Phaseolus vulgaris*), evidence for large variations in use and translocation of P (Lindgren et al., 1977), K (Gabelman and Gerloff, 1978), Zn (Polson and Adams, 1970), and Ca (Quintana et al., 1996a) have been demonstrated. Plant species have evolved differently based on the selection pressure associated with high and low Ca in the environ-

ment (Clarkson, 1965), resulting in diverse frequencies of Ca-enhancing genes among genotypes. A recent study has shown that variability for pod Ca concentration in snap beans could be related to differences in transport of Ca via root pressure (Quintana et al., 1997).

Recent studies have shown that genetic differences for Ca concentration in bean pods exist (Quintana et al., 1996a) and have suggested that breeding is the best way to enhance pod Ca accumulation in beans (Miglioranza et al., 1997; Quintana et al., 1998). However, knowledge of the genetic mechanisms controlling Ca accumulation in snap beans is limited (Quintana et al., 1996a). The objective of this study is to estimate and compare the genetic variances and heritabilities associated with pod Ca concentration between two snap bean populations.

Materials and Methods

PLANT MATERIAL. Two snap bean populations high in pod Ca concentration and sharing a common parent were selected for this investigation from the snap bean elite synthetic (SBES) population. The snap bean populations (CA2 and CA3) were developed and taken to the F$_4$ generation via single seed descendent method (Poehlman and Sleper, 1996), by allowing to self-pollinate two high Ca individuals CA2 ('Evergreen' x 'Top Crop') and CA3 ('Evergreen' x 'Slimgreen'), selected from SBES population (Quintana et al., 1996a). Sixty F$_4$ families were randomly selected from each population for evaluation. The original SBES population was created by random mating among 50 snap bean breeding lines and cultivars (J. Nienhuis and K. Kmiecik, unpublished data). The SBES population was developed and is currently maintained at the Dept. of Horticulture, Univ. of Wisconsin, Madison. Cultivars and breeding lines used as parents in the SBES population were selected to maximize genetic variation for an array of traits including pod yield, disease resistance, pod quality, earliness, and plant architecture.

EXPERIMENTAL DESIGN. Field experiments were conducted at the University of Wisconsin Research Station at Hancock, Wis.

For each population, 60 F_4 families and 4 common checks ('Evergreen', 'Slimgreen', 'Top Crop', and 'Labrador') were planted in an 8×8 double lattice design replicated in 1995 and 1996. The soil at Hancock is characterized as Plainfield loamy sand with $\approx 1\%$ organic matter. Soil analyses revealed a pH of 6.1, 88 ppm of P_2O_5, 107 ppm of K_2O, and 580 ppm of Ca in 1995; and a pH of 6.4, 72 ppm of P_2O_5, 75 ppm of K_2O, and 500 ppm of Ca for 1996. No additional Ca was added to the soil. Because the efficiency of the lattice design was less than 105% when compared to randomized complete blocks (e.g., mean squares for blocks is less than intrablock error), the experiments were analyzed as randomized complete blocks with two replications each year (Cochran and Cox, 1950).

STATISTICAL ANALYSES. A pooled analysis of variance (ANOVA) across years was made on the data from each population using SAS (SAS Institute, Cary, N.C.). Estimates of variance components were obtained by pooling the sums of squares over years (Hallauer and Miranda, 1981). An estimate of additive genetic variance (σ^2_A) was calculated from the genotype variance component (σ^2_g), assuming gene frequency of 0.5 within population. Narrow sense heritability estimates (h) on an entry mean basis were calculated as the ratio of σ^2_A/σ^2_P, where $\sigma^2_P = \sigma^2_e/ry + \sigma^2_{gy}/y + \sigma^2_g$ (Hallauer and Miranda, 1981), e = experimental error, g = genotypes within population, r = replications, and y = number of years. To compare variances between populations, a Bartlett test for homogeneity of variance among mean Ca concentration of genotypes was done between populations (Steel et al., 1996). In addition, the Shapiro-Wilk test for distributions was used to assess normality of both populations' data (Shapiro and Wilk, 1965).

PLANT CULTURE AND SAMPLING. During 1995, bean seeds were planted on 22 June (CA2) and 5 July (CA3); and plantings occurred on 7 June (CA2 and CA3) in 1996. Planting depth was ≈ 5 cm. Within each 1.02-m-row plot, 20 seeds were seeded. Plots were thinned (≈ 15 d after planting) to stands of 10 seedlings per row. Spacing between rows and blocks was 91 cm. Thus, the total area of each plot was 0.93 m^2. Cultural practices included preplant incorporation of the herbicide Trifluralin (1.1 $kg \cdot ha^{-1}$) and cultivation as needed to control weeds, a single side dressed application (100 $kg \cdot ha^{-1}$) of 33.5N–0P–0K roughly 15 d after planting, insecticide (Acephate) applications at a rate of 1 $kg \cdot ha^{-1}$ as required for leafhoppers (*Empoasca fabae*) control, and an irrigation schedule of 12.5 mm per week until harvest (Binning et al., 1998). Flowering began ≈ 40 to 45 d after planting in both populations. Beans reached maturity and were hand harvested 55 d (1995) and 61 d (1996) after planting. To provide pod Ca concentration estimates unbiased by pod diameter (Quintana et al., 1996b), only pods of commercial sieve size no. 4 (8.3 to 9.5 mm in diameter) were harvested (Robinson et al., 1963). A pooled sample of ≈ 15 sieve size no. 4 pods was then taken from each plot for Ca determinations.

LABORATORY ANALYSIS. The harvested pods were oven-dried at 60 to 65 °C for 48 h and then ground to pass a 10-mesh screen. A 0.05-g sample for each treatment was weighed and placed into a 10-mL glass beaker. Samples were dry-ashed at 450 °C for 5 h. Calcium was extracted by adding 5 mL of 2 N HCl to the cooled samples. The solution was then poured through Whatman no. 540 filter paper and collected into a 50-mL volumetric flask. The filter paper was rinsed with two to three volumes of distilled–deionized water, and 10 mL of 0.2 N HCl containing 10,000 ppm lanthanum (as $LaCl_3$) was added to the extracted Ca to suppress mineral interferences. Total volume was brought to 50 mL with distilled–deionized water and readings were made using atomic absorption spectrophotometry (AAS) (model Spectra AA-20; Varian Techtron Pty. Limited, Mulgrave Victoria, Australia).

Results and Discussion

ENVIRONMENTAL ATTRIBUTES. Years differed for pod Ca concentration in both populations (Table 1). In 1995, mean pod Ca concentration for CA2 (5.92 $mg \cdot g^{-1}$ dry weight) and CA3 (5.35 $mg \cdot g^{-1}$ dry weight) were 11% to 30% higher than those in 1996 (Table 1). Previous studies have reported that increasing soil Ca beyond plant sufficiency levels does not result in higher pod Ca concentration in snap beans (Allaway, 1984; Miglioranza et al., 1997; Quintana et al., 1998); therefore, year differences for pod Ca concentration might be due to variation in temperature, rainfall or other environmental factors. The 1995 growing season was hotter [23% more heat units (base 10 °C)] and wetter (43% more accumulated precipitation) than in 1996 (data not shown). These differences in heat units and precipitation between years are particularly important because pod Ca accumulation in snap beans is associated with Ca transport via root pressure (Quintana et al., 1997), and lower temperature and soil moisture can significantly reduce root pressure (Kramer, 1983).

DIFFERENCES BETWEEN POPULATIONS. Both populations were normally distributed ($P > 0.10$) (Shapiro-Wilk test for normality). A relatively wide range of values for pod Ca concentration levels were found in both snap bean populations (Fig. 1). A *t* test performed in the population means for pod Ca concentration revealed that populations did not differ ($P = 0.241$). The mean pod Ca concentration for CA2 was 5.24 $mg \cdot g^{-1}$ dry weight and for CA3 was 5.09 $mg \cdot g^{-1}$ dry weight (Fig. 1). These values are in accordance with an overall mean pod Ca concentration of 5.07 $mg \cdot g^{-1}$ dry weight found in our previous studies (Quintana et al., 1996a).

Pod Ca concentration values ranged from 4.22 to 6.40 and from 3.82 to 6.80 $mg \cdot g^{-1}$ dry weight in CA2 and CA3, respectively (Table 1). Snap bean commercial cultivars used as checks ('Top

Table 1. Analysis of variance, year means, variance components and heritability (h^2) for pod calcium concentration in two F_4 populations CA3 ('Evergreen' $\times$ 'Slimgreen') and CA2 ('Evergreen' $\times$ 'Top Crop') of snap beans.[z]

Source	df	MS, ANOVA Pod Ca concn ($mg \cdot g^{-1}$ dry wt)	
		CA2	CA3
Year (Y)	1	108.292***	11.397***
Replication (year)	2	0.262	0.016
Genotype (G)	59	0.866***	0.890***
Y × G	59	0.449	0.442**
Error	114	0.403	0.329
Year means			
1995		5.92	5.35
1996		4.56	4.83
Variance component[y]			
σ^2_g (G)		0.104	0.112
σ^2_{gy} (G × Y)		0.023	0.057
σ^2_p (phenotypic)		0.216	0.223
Heritability[x]			
h^2		0.48	0.50

[z]Measurements made in sieve size pods no. 4 (8.3 to 9.4 mm in diameter).
[y]Phenotypic variance (σ^2_p) = $\sigma^2_e/ry + \sigma^2_{gy}/y + \sigma^2_g$, (where r = number of replications and y = number of years, respectively.
[x]Heritability on an entry mean basis = σ^2_g/σ^2_p.
,*Significant at $P = 0.01$ or 0.001, respectively.

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variance between populations was not significant ($P>0.10$), suggesting that variances between populations were not different and could be pooled for further analyses.

VARIABILITY AMONG GENOTYPES WITHIN POPULATIONS. Significant differences were observed for pod Ca concentration among the 60 genotypes within each of the populations (Table 1). Variability for this trait in snap beans was consistent with the results of previous studies in snap beans (Quintana et al., 1996a) and other crops including tomatoes (Giordano et al., 1982), corn (Clark, 1978), soybeans (Kleese, 1967), barley (Young and Rasmusson, 1966), wheat (Rasmusson et al., 1971), sorghum (Kawaski and Moritsugu, 1979), and sunflower (De La Guardia et al., 1980). Year × genotype interaction for pod Ca concentration was not detected in CA2, but was significant in CA3 although the mean squares were approximately half those found for differences among genotypes (Table 1). The results suggest that possibly genotypic expression across environments is stable for this trait (pod Ca concentration) in snap beans, which is in accordance with the results from previous studies (Quintana et al., 1996a). However, more work in this area needs to be done in order to be certain.

PHYSIOLOGICAL BASIS. Since Ca is not mobile in the phloem (Raven, 1977) and not redistributed within the plant (Biddulph et al., 1959), variability for pod Ca concentration in snap beans must be caused by differences in direct Ca uptake (Barber, 1995). When low transpiration conditions exist in the environment (especially at night and when soil is moist, warm, and well aerated) healthy roots can behave as osmometers producing root pressure that can cause guttation in leaves (Kramer, 1983). Recent research has shown that some of the alteration for pod Ca concentration among snap bean genotypes could be due to differences in Ca uptake via root pressure (Quintana et al., 1997), causing these genotypes to show variability for this trait. This suggests that snap bean genotypes that are higher pod Ca accumulators should exhibit higher xylemic sap and Ca transport via root pressure.

HERITABILITY AND GENETIC VARIANCE. The ANOVA reveals genetic variability for pod Ca concentration among snap bean genotypes for both populations (Table 1). Despite large differences between years, no year × genotype interactions were found (Table 1). Genetic variance components (σ^2_g) are 4.5 and 2 times the magnitude of their respective environmental variance components (σ^2_{gy}) in populations CA2 and CA3, respectively (Table 1). This facilitates development of high pod Ca bean lines via plant breeding because evaluation and selection could be probably done under one environment while maintaining phenotypic resemblance (high pod Ca concentration) for this trait over other environments.

Narrow sense heritability values for pod Ca concentration in snap beans were 0.48 for CA2 and 0.50 for CA3, which resembled the heritability values (0.50 ± 0.03) for this trait found in earlier studies (Quintana et al., 1996a). Heritability values (Table 1) were several times higher than the values reported for inheritance of Ca use and efficiency in wheat and barley ($h^2 < 0.10$) (Rasmusson et al., 1971) and slightly lower than the average of that in tomato (h^2

Fig. 1 Mean pod Ca concentration of pods size no. 4 for two F_4 populations—CA3 (EV × SG) and CA2 (EV × TC)—and four commercial cultivars—Evergreen (EV), Top Crop (TC), Labrador (LB), and Slimgreen (SG)—of snap beans over 2 years (1995 and 1996).

Crop', 'Slimgreen', 'Evergreen', and 'Labrador') in both experiments showed pod Ca concentration values relatively consistent across experiments (Fig. 1). 'Top Crop' (5.64 mg·g^{-1} dry weight) and 'Slimgreen' (5.63 mg·g^{-1} dry weight) had the highest mean pod Ca concentrations, followed by 'Evergreen' (5.43 mg·g^{-1} dry weight) and 'Labrador' (3.91 mg·g^{-1} dry weight), which ranked lowest among the check cultivars (Fig. 1). Several genotypes in each of these populations (CA2 and CA3) showed pod Ca concentrations 7% to 13% higher than the best of the checks ('Top Crop' and 'Slimgreen'). In contrast, Ca increased only 3% to 4% when soil fertilizers were used to augment pod Ca in snap beans (Quintana et al., 1998). These findings suggest that pod Ca concentration in snap beans could be increased through plant breeding.

The standard deviations (CA2 = 0.47 and CA3 = 0.54 mg·g^{-1} dry weight) were approximately half those in our previous study (0.92 mg·g^{-1} dry weight); indicating that pod Ca concentrations among genotypes clustered close to the population mean, apparently because individuals within each population for this study originated from crosses to a common parent ('Evergreen') rather than among numerous parents as occurred in our previous work (Quintana et al., 1996a). The Bartlett test for homogeneity of

≈ 0.59) (Li and Gabelman, 1990). Furthermore, the heritability estimates for pod Ca concentration found in this study suggest that the heritable variability for this trait in snap beans justifies the use of these genotypes in breeding programs targeted to enhance the accumulation of Ca in bean pods for human nutrition purposes.

Conclusion

The two populations studied in this experiment were derived from crosses to a common parent cultivar, Evergreen. The genetic variances and means for pod Ca concentration did not differ between populations. Heritability estimates (h^2) were similar in both populations indicating that gain from selection would be comparable. Environmental variance components in both snap bean populations were relatively low when compared to genetic variance components, denoting that environmental factors should not be of major concern in breeding programs targeted to increase pod Ca concentration in snap beans. Several genotypes had pod Ca concentrations superior to those observed in the best of the commercial cultivars evaluated in this study. Breeding can be successful in enhancing Ca accumulation in pods, and pod Ca accumulation can be increased even in high Ca snap bean populations. Snap beans posses high Ca bioavailability when compared to other foods. High consumption rates and economic significance make snap beans a vegetable with remarkable importance to the human diet. Its impact as an additional source of Ca for humans could be substantial if Ca levels can be significantly enhanced.

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