J. Amer. Soc. Hort. Sci. 121(5):789–793. 1996.

Variation in Calcium Concentration among Sixty S_1 Families and Four Cultivars of Snap Bean (*Phaseolus vulgaris* L.)

Juan M. Quintana[1], Helen C. Harrison[2], James Nienhuis[3], and Jiwan P. Palta[2]
Department of Horticulture, University of Wisconsin–Madison, Madison, WI 53706

Michael A. Grusak[4]
U.S. Department of Agriculture, Agricultural Research Service, Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, 1100 Bates Street, Houston, TX 77030–2600

Additional index words. atomic absorption spectrophotometer, nutrient absorption, plant nutrition, human nutrition

Abstract. **To assess nutritional potential, pod yield, and Ca concentration of pods and foliage were determined for a snap bean population, which included sixty S_1 families plus four commercial varieties. The experimental design was an 8×8 double lattice, repeated at two locations (Arlington and Hancock, Wis.). Snap beans were planted in June 1993 and machine harvested in August 1993. Calcium analyses were made using an atomic absorption spectrophotometer. Significant differences were detected in pod Ca concentration and yield among the S_1 families. Pod size and Ca concentration were inversely correlated ($R^2 = 0.88$). Distinct differences between the locations were not observed, and higher Ca genotypes remained high regardless of location or pod size. Low correlation ($R^2 = 0.21$) between pod and leaf Ca concentration was found. Pods of certain genotypes appeared to have the ability to import Ca more efficiently than others, but this factor was not related to yield.**

Green leafy vegetables rank as relatively good sources of Ca (Macrae et al., 1993) in addition to milk and dairy products (Ensminger and Robson, 1983). Among 39 major fruit and vegetables analyzed for nutritional value, snap beans (*Phaseolus vulgaris*) ranked third for Ca content (Stevens, 1974). The snap bean is one of the major vegetables grown in the United States; in 1992 it was fourth among all vegetables, with a production value of $239,033,000 (National Agricultural Statistics Service, 1992). Because about 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 Ca.

Previous nutritional studies involving snap beans grown in the United States have revealed a wide range of concentrations for the 18 elements analyzed, including Ca (Mills and Jones, 1979). Crop variability in acquisition and use of essential elements is common (Gerloff et al., 1966). This study was designed to determine 1) the extent of genetic variability in pod Ca concentration in a breeding population of snap bean and 2) the relationship between pod Ca concentration and pod size and leaf Ca concentration.

Received for publication 19 Dec. 1995. Accepted for publication 14 May 1996. Research conducted at the Univ. of Wisconsin–Madison, Madison Wis. We appreciate the technical assistance of Kenneth Kmiecik and the constructive comments of Dennis P. Stimart, Jack E. Staub, and two anonymous reviewers. Research supported by the Graduate Research Committee at the Univ. of Wisconsin–Madison, the College of Agricultural and Life Sciences, Hatch Act funds and the Mexican government. This work has also been funded in part with federal funds from the U.S. Dept. of Agriculture–Agricultural Research Service under cooperative agreement 58–6250–1–003 to M.A.G. The contents of this publication do not necessarily reflect the views or policies of the U.S. Dept. of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. government. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]Graduate student.
[2]Professor.
[3]Associate professor.
[4]Research plant physiologist.

Materials and Methods

Plant material. A snap bean elite synthetic (SBES) population was developed by random mating among 50 snap bean breeding lines and cultivars (J. Nienhuis, unpublished data). The SBES population is maintained at the Dept. of Horticulture, Univ. of Wisconsin, Madison. Breeding lines and cultivars included as parents in the SBES population were chosen to maximize genetic variation for an array of traits, including: earliness, pod yield, pod quality, plant architecture, and resistance to pathogens. Sixty random hybrids from the SBES population were allowed to self-pollinate to produce S_1 families (F_2 populations) for evaluation in this study.

Experimental design and analysis. The sixty S_1 families and four cultivars (Hystyle, Labrador, Evergreen, and BBL94) were planted in an 8×8 double lattice design repeated at two locations—the Hancock and Arlington experimental stations—in Wisconsin. Soil analysis taken preplant at both locations revealed that the soil at the Hancock Experimental Station was characterized as a sandy soil with a pH of 6.5 to 7.0 with 125 ppm of P_2O_5, 175 ppm of K_2O, and 650 ppm of Ca, and the soil at the Arlington Experimental Station was characterized as a silt loam, pH 6.5 to 7.0 with 31 ppm of P_2O_5, 105 ppm of K_2O, and 1950 ppm of Ca. No additional Ca was added to the soil at either location. Because the efficiency of the lattice design when compared to randomized complete block was <105% (e.g., variation between blocks in lattice was not significant), the experiments were analyzed as a randomized complete-block design with two replications at each location. In so doing, the variation among the blocks of the lattice design is included in the experimental error (Boyce, 1945).

A pooled analysis of variance (ANOVA) was performed on the data by pooling the sums of squares over locations to obtain estimates of variance components (Hallauer and Miranda, 1981). Genetic components of variance and covariance were obtained by setting observed mean squares or cross products equal to expected values and solving for the desired component (Hallauer and Miranda, 1981). Total genetic variance was used for the calcula-

tion of genotypic correlations among traits. An estimate of the additive genetic variance (σ^2_A) was obtained from the family component of variance (σ^2_f) , under the assumption that gene frequencies were 0.5 within and between each S_1 family (Stuber, 1970). Narrow sense heritability estimates (h^2) on an entry mean basis were calculated as the ratio of σ^2_A/σ^2_P, where $\sigma^2_P = \sigma^2_e/4 + \sigma^2_{fl}/2 + \sigma^2_f$ (Hallauer and Miranda, 1981), and where e = experimental error, f = number of families, and l = number of locations.

Plant culture. Snap bean seeds were planted on 16 June 1993 at Arlington and 23 June 1993 at Hancock, with each plot consisting of one row 1.02 m long. Twenty seeds were double seeded and thinned 2 weeks after planting to stands of ten seedlings per row, 10.2 cm apart. Rows were spaced 91 cm apart and blocks were spaced 60 cm apart. Standard cultural practices were followed (Binning et al., 1995). These practices included preplant incorporation of herbicide (Treflan), two cultivations to control weeds (3 and 6 weeks after planted), and a single fertilizer application (33N–0P–0K) at rate of 100 kg·ha^{-1}. Most genotypes were mature commercially (LeBaron, 1974) at the time of harvest, 23 and 30 Aug. 1993 at Arlington and Hancock, respectively. All S_1 families and commercial cultivars were machine harvested. All genotypes matured at about the same time. At harvest, most pods were full and seeds were small, which corresponded to its R_5 to R_6 plant life stages (LeBaron, 1974).

Postharvest procedure. Pods were weighed and separated into sieve size grades by a Chisholm-Ryder double adjustable bar-type grader (Peck et al., 1989). Sieve sizes for small pod diameters ranged from 5.8 to 8.2 mm, for medium pods 8.3 to 9.5 mm, and for large pods >9.5 mm in diameter (Mullins and Straw, 1988). Before being weighed and graded, the pods were stored in a cold, dark room to maximize nutrient and water retention (Yamaguchi, 1983). For three of the four commercial cultivars (Evergreen, Hystyle, and Labrador), small, medium, and large pods and all foliage were weighed, graded, and saved for Ca determinations (BBL94 was excluded due to missing plots). To ensure proper comparison for Ca concentration among genotypes, only medium size pods were saved for the 60 S_1 families derived from the SBES population. Medium pods are used most commonly in the processing industry and were the most abundant at harvest (Weidman and Viets, 1993).

Laboratory analyses. After being weighed and graded, samples were oven-dried at 60 to 65 °C for 1 week. Again, samples were weighed (dry weight determination) and ground in a Wiley mill to pass a 10-mesh screen. Two duplicate 0.15-g samples for each treatment were weighed and placed into 10-mL glass beakers. Samples were dry ashed in a muffle furnace at 450 °C for about 5 h. When the samples were cooled, the Ca was extracted by adding 5 mL of 2 N HCl to dissolve the ash. This solution was poured through Whatman no. 540 filter paper and collected into a 50-mL volumetric flask. Filter paper was rinsed with two to three volumes of distilled water to ensure that all Ca was extracted from the ash. Finally, 10 mL of 0.2 N HCl containing 10000 ppm lanthanum (as $LaCl_3$) was added to the Ca extract to overcome chemical interferences (Macrae et al., 1976), and the total volume was brought to 50 mL with deionized water. Calcium analyses and readings were made with an atomic absorption spectrophotometer (model Spectra AA–20; Varian Techtron Pty. Limited, Mulgrave Victoria, Australia) (Li and Gabelman, 1990).

Results and Discussion

Population attributes. The Ca concentrations (medium pods) for the 64 genotypes averaged among the two locations are shown in Fig. 1. The four commercial cultivars (Labrador, BBL94, Hystyle, and Evergreen) had pod Ca concentrations within the range of values for the SBES genotypes. Most genotypes had Ca concentration values of about 5 mg·g^{-1} dry weight, which was similar to previous studies (Ensminger and Robson, 1983; U.S. Dept. of Agriculture, 1984). The standard deviation for this distribution was 0.92, and values ranged from a low of 3.5 to high of 6.6 mg Ca/g dry weight. One of the cultivars used as a check, 'Labrador', was among the lowest in pod Ca concentration (4.6 mg Ca/g dry weight). In contrast, 'Hystyle' was similar in pod Ca to the best S_1 families (6.6 mg Ca/g dry weight). The bell shaped curve of this graph (Fig. 1) suggests a fairly normal distribution of pod Ca concentration and a relatively wide range of values for pod Ca concentration among the genotypes.

Quantitative variability between families. The results demon-

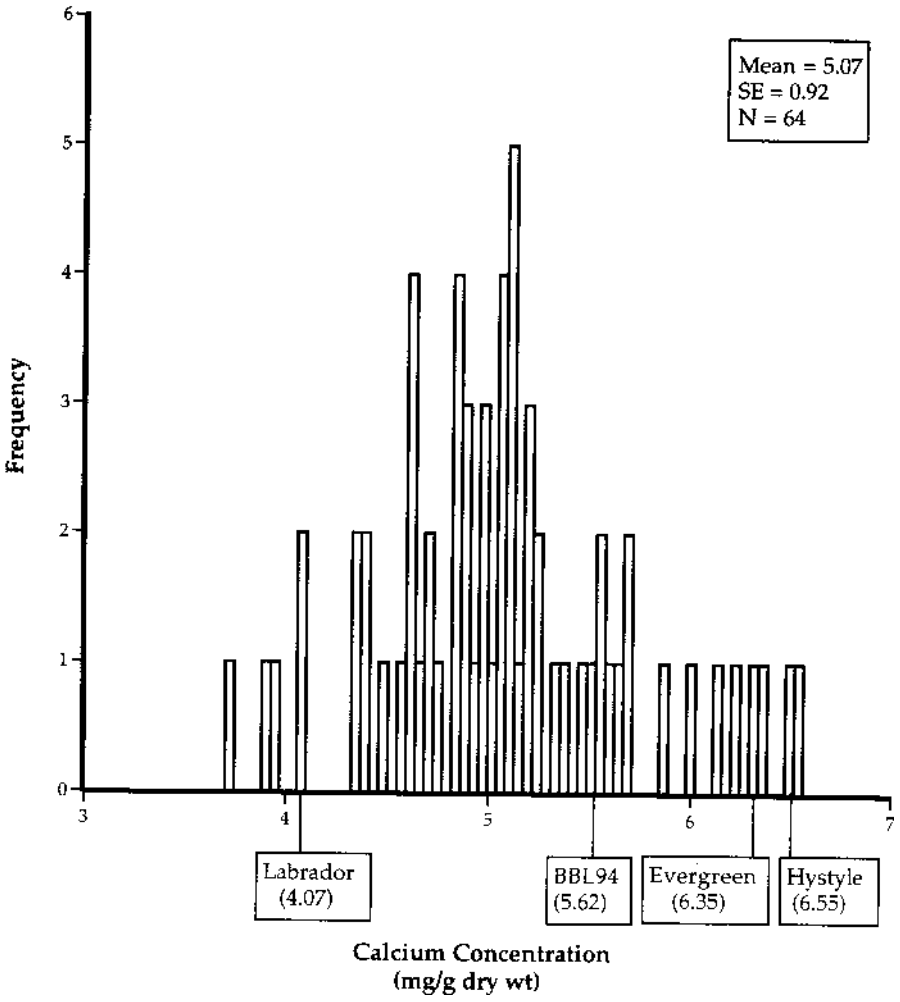

Fig. 1. Mean calcium concentration of medium-sized pods for the sixty S_1 families and four commercial cultivars of snap beans over two locations.

Table 1. Analysis of variance, locations means, variance components and heritability (h^2) for medium pod yield (Medium), total pod yield (Total), and pod calcium concentration (Ca) in the Snap Bean Elite Synthetic population.[z]

		Mean squares		
		ANOVA		
			Pod yield ($g \cdot m^{-2}$)	
Source	df	Ca ($mg \cdot g^{-1}$ dry wt)	Total	Medium
Location (L)	1	0.056	457.742**	105.100
Rep (L)	2	0.048	28.276	6.804
Family (F)	59	1.284**	74.432**	29.599**
L × F	59	0.641	54.797*	26.348*
Error	117	0.514	32.596	17.847
			Location means	
Arlington		5.26	1147	325
Hancock		4.80	465	328
			Variance component	
σ^2 f (F)		0.161	4908.8	812.8
σ^2 fl (F × L)		0.064	11100.5	4250.5
σ^2 p[y] (phenotypic)		0.322	18608.0	7399.8
			Heritablity	
h^{2x} ± SE		0.5 ± 0.03	0.26 ± 0.03	0.11 ± 0.03

[z]Small (≤8.2 mm diameter), medium (8.3–9.5 mm diameter) and large (>9.5 mm diameter), sieve size pods.
[y]Phenotypic variance (σ^2p) = σ^2e/rl + σ^2fl/l + σ^2f, (where r = number of replications and l = number of locations, respectively.
[x]Heritability on an entry mean basis = σ^2f/σ^2p.
*,**Significant at P = 0.1 and 0.01 respectively.

strate significant differences among the 60 SBES families for all variables analyzed (medium pod Ca concentration, fresh weight of medium size pods, and fresh weight yield). Since Ca in plants is transported in the xylem and is not redistributed (Palta, 1996), our results presented in Table 1 suggests that genotypes with high pod Ca concentrations were more efficient at importing Ca than those having low pod Ca concentrations, regardless of location (Arlington or Hancock) or replication. These results are consistent with the observed genetic variation for the efficiency of Ca use in tomatoes (Li and Gabelman, 1990) and differences among genotypes in the acquisition of minerals like N in tomato (O'Sullivan et al., 1974); K in snap beans (Shea et al., 1967); and P in tomatoes (Figdore et al., 1989) and snap beans (Fawole et al., 1982), respectively.

Pod Ca concentration did not differ significantly between the Arlington and Hancock locations (Table 1). Pod Ca concentration averaged among all treatments was 5.3 mg Ca/g dry weight in Arlington, whereas it was 4.8 mg Ca/g dry weight in Hancock. Apparently the environmental conditions and soil characteristics of each location did not have a significant effect on the ability of each snap bean family to absorb and distribute Ca; however, soil fertility has been shown to influence the elemental content of snap bean plants (Leggett et al., 1975).

Relationship between pod size and pod Ca concentration. Pod Ca concentration for the three commercial cultivars (averaged over two locations) was plotted against the mean pod diameter for the three pod sizes: small, medium, and large (Fig. 2). Although mean pod diameters are depicted in this figure, these values are based on mean values of the grade sizes (i.e., small, medium, large) and not on actual mean sizes of individual pods harvested and separated. Nevertheless, an inverse relationship exists between pod Ca concentration and pod size (Fig. 2), indicating that Ca concentration decreases as pods mature. In the case of 'Evergreen', as pod size increased from 7 (mean of small sieve size) to 10.7 mm (mean of large sieve size), the corresponding Ca concentration decreased from 9.9 to 5.9 mg Ca/g dry weight. Similar results have been reported for snap bean Ca concentrations (Mills and Jones, 1979; Mix and Marschner, 1976). It also was observed that high Ca cultivars (e.g., Evergreen) were high regardless of pod size, relative to equivalent pod sizes in the other cultivars. In addition, the three cultivars share the same estimated slope but have different intercepts (Fig. 2). This suggests that the negative relationship between sieve size and pod Ca concentration may be a consistent and predictable trend in snap beans.

Fig. 2. Linear regressions of calcium concentration for small, medium, and large pods for three commercial snap bean cultivars harvested from Hancock and Arlington, Wis.

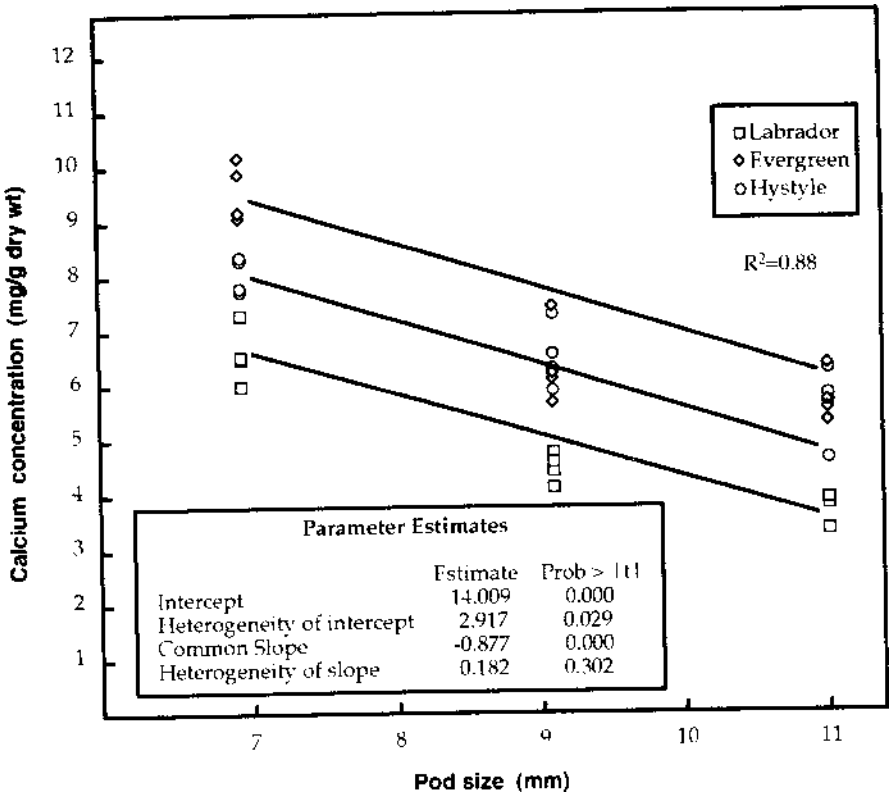

Table 2. Phenotypic (above diagonal) and genotypic (below diagonal) correlations among calcium concentration in medium-sized pods, foliage calcium concentration, total yield, and yield of medium-sized pods.[z]

	Ca concentration		Yield	
	Foliage	Pod	Total	Medium
Pod Ca concentration[y]	0.216	---	-0.078	-0.047
Total yield	---	0.001	---	0.644**
Medium pod yield	---	-0.010	0.000	---

[z]Small (≤8.2 mm in diamter), medium (8.3–9.4 mm in diamter), and large (>9.5 mm in diamter) sieve size pods.
[y]Calcium concentration in medium-sized pods only.
**Significant at $P = 0.01$.

Relationship between foliage and pod Ca concentration. Concentration of Ca in the foliage was not significantly correlated with Ca in pods (Table 2). Thus, genotypes with higher pod Ca concentrations did not necessarily exhibit higher Ca concentrations in foliage. Foliage Ca concentrations for the three commercial cultivars (Evergreen, Hystyle and Labrador) were similar, averaging 36.9 and 24 mg Ca/g dry weight at the Arlington and Hancock sites, respectively. These values were 3- to 6–fold higher than the Ca concentrations of medium pods from the same cultivars. It appears that, because leaves were transpiring constantly and were present on the plant for a longer period of time, they were able to import more total Ca than pods. These results suggest that genotypes may not differ in their total net absorption of Ca, but rather in their Ca use and internal distribution.

Genetic variance and heritability. Although the Arlington Experimental Station had greater soil Ca compared to Hancock Experimental Station, pod Ca concentration was not different between the two locations (Table 1). The results suggest that higher soil Ca does not necessarily result in higher pod Ca concentration. Perhaps Ca levels were not low enough in either location to cause a Ca deficiency (Walsh, 1973). Although, total yield was greater at Arlington than Hancock, no difference was detected for the yield of medium sieve size pods. The data also indicate that beans at the Arlington location were harvested when more large diameter pods were present.

The ANOVA revealed significant variation among the 60 S_1 families for medium pod Ca concentration, and total and medium pod yield (Table 1). This result is consistent with observed genetic variances for certain nutrient efficiencies in various vegetable crops (Fawole et al., 1982; Figdore et al., 1989; O'Sullivan et al., 1974; Shea et al., 1967). Significant family × location interactions were observed for total and medium pod yield, but not for medium pod Ca concentration. The consistent rank among families for medium pod Ca over the two locations, and the observation that the family component of variance (σ^2_f) is over twice the magnitude of the family × location component of variance (σ^2_{fl}), suggests that selection may be effective at only one location. In contrast, the large family × location interaction and variance components suggest that ranking of yield for the S_1 families was not consistent over locations. This is also reflected in the relatively low heritabilities associated with total (0.26 ± 0.03) and medium pod yield (0.11 ± 0.03) compared to Ca content (0.50 ± 0.03).

A significant phenotypic correlation was observed only between total yield and yield of medium pods (0.644). However, all genetic correlations were near 0 (Table 2). This suggests that selection for increased pod Ca will not result in correlated responses for either increased or decreased total or medium pod yield.

Results of the present study demonstrate the existence of genetic variations for Ca accumulation in snap bean pods, thus showing promising potential for the improvement for this trait through breeding. Neither genetic nor physiological factors that lead to such results are fully understood yet. Furthermore, these materials should provide a valuable resource for understanding physiological mechanisms associated with variation in calcium accumulation. This study is the first step toward the eventual goal of improving the nutritional quality of snap beans with respect to Ca based on selection.

Literature Cited

Binning, L.K., C.M. Boerboom, L.G. Bundy, D. Curwen, E.T. Gritton, H.C. Harrison, H.J. Hopen, K.A. Kelling, S.E.R. Mahr, B.A. Michaelis, P.J Schmidt, W.R. Stevenson, W.F. Tracy, J.L. Wedberg, and J.A. Wyman. 1995. Commercial Vegetable Production in Wisconsin. University of Wisconsin Extension. Bul. A3422 Univ. of Wisconsin, Madison.

Boyce, S.W. 1945. The efficiency of lattice designs. N.Z. J. Sci. and Technol. 27:270–275.

Ensminger, E. and Robson K. 1983. Foods and nutrition encyclopedia. vol. 1. Pegus Press, Clovis, Calif. p. 169–179, 302, 966–969.

Fawole, I., W.H. Gabelman, and G.C. Gerloff. 1982. Heritability of efficiency in phosphorus utilization in beans (*Phaseolus vulgaris* L.) grown under phosphorus stress. J. Amer. Soc. Hort. Sci. 107:94–97

Figdore, S.S., W.H. Gabelman, and G.C. Gerloff. 1989. Inheritance of potassium efficiency, sodium substitution capacity and sodium accumulation in tomatoes grown under low-potassium stress. J. Amer. Soc. Hort. Sci. 114:322–327.

Gerloff, G.C., D.G. Moore, and J.T. Curtis. 1966. Selective absorption of mineral elements by native plants of Wisconsin. Plant Soil. 25:293–405.

Hallauer, A.R. and J.B. Miranda. 1981. Quantitative genetics in maize breeding. Iowa State Univ. Press, Ames.

Le Baron, M.J. 1974. Developmental stages of the common bean plant. Current Info. Ser. 228. Univ. of Idaho.

Leggett, G.E., D.T. Waster Mann, and M.J. Le Baron. 1975. A survey of nutrient status of leaves grown in Southern Idaho. In : Proc. 26th Annu. Pacific. North West Fert. Conf. Salt Lake City, Utah. p. 131–138.

Li, Yin–ming and W.H. Gabelman. 1990. Inheritance of calcium use efficiency in tomatoes grown under low–calcium stress. J. of Amer. Soc. Hort. Sci. 115:835–838.

Macrae, R., R.K. Robinson, and M.J. Sadler. 1993. Encyclopedia of food science, food technology and nutrition. vol. 1. Academic Press, San Diego California. p. 585–589.

Mix, G.P. and H. Marschner. 1976. Redistribution of calcium in bean fruits during seed development. Z. Pflanzenphysiol. 80:354–366.

Mills, H.A. and J.B. Jones, Jr. 1979. Elemental content of frozen snap bean fruit. HortScience 14:268–269.

Mullins, C.A. and A.R. Straw. 1988. Production of snap bean as affected by soil tillage method and row spacing. J. Amer. Soc. Hort. Sci. 113:667–669.

National Agricultural Statistics Service. 1992. Bul. TVS–259.

O'Sullivan, J., W.H. Gabelman, and G.C. Gerloff. 1974. Variation in efficiency of nitrogen utilization in tomato (*Lycopersicon esculentum*) grown under nitrogen stress. J. Amer. Soc. Hort. Sci. 99:543–547.

Palta, J.P. 1996. Role of calcium in plant responses to stresses: Linking basic research to the solution of practical problems. In: M. Wisniewski (eds.). Recent advances in plant responses to stress: Bridging the gap between science and technology. HortScience. 31:51–57.

Pao, E., K.H. Fleming, PM. Guenther, and S.J. Mickle. 1982. Foods commonly eaten by individuals. USDA/HNIS. Home Econ. Rpt. 44. p. 408–409.

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. 24:619–623.

Shea, P.F., W.H. Gabelman, and G.C. Gerloff. 1967. The inheritance of efficiency in potassium utilization in snap beans (*Phaseolus vulgaris* L.). Proc Amer. Soc. Hort. Sci. 91:286–293.

Stevens, M.A. 1974. Nutritional quality of fresh fruits and vegetables. Futura Publishing, Mt. Kisco, N.Y. p. 87–110.

Stuber, C.W. 1970. Estimation of genetic variances using inbred relatives. Crop Sci. 10:129–135.

U.S. Department of Agriculture. 1984. USDA agricultural handbook series 8. Composition of foods: Vegetables and vegetable products—Raw, processed prepared. Nutr. Monitoring Div., Human Nutr. Info. Serv., U.S. Dept. of Agr., Hyattsville, Md.

Walsh, L.M. 1973. Soil and Applied Calcium. Univ. of Wisconsin, Madison, Ext. Bul. A2523.

Weidman, R. and J. Viets. 1993. Evaluation of green bean varieties. Univ. of Wisconsin Peninsular Agr. Res. Sta., Sturgeon Bay. Res. Rpt. p. 40.

Yamaguchi, M. 1983. World vegetables principles, production and nutritive value. Ellis Horwood Limited Publishers, England. p. 39–41.