Xylem Flow Rate Differences Are Associated with Genetic Variation in Snap Bean Pod Calcium Concentration

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ABSTRACT. Two commercial snap bean (*Phaseolus vulgaris* L.) cultivars (Hystyle and Labrador) that differ in pod Ca concentration were grown aeroponically to assess physiological factors associated with these differences. Xylem flow rate, Ca absorbed, and Ca concentration in sieve sap and pods (all and commercial size no. 4) were measured. Flow rate, Ca absorption and pod Ca concentration, but not sap Ca concentration, differed between cultivars, and this suggests that genetic variability in pod Ca concentration is caused mainly by differences in flow rate, rather than differences in sap Ca concentration. 'Hystyle' showed 1.6 times greater flow rate, 1.5 times greater pod Ca concentration, and 1.7 times greater Ca absorbed than 'Labrador'. Flow rate correlated positively with Ca absorbed ($R = 0.90$), Ca concentration in pods of size no. 4 ($R = 0.55$), and total pods ($R = 0.65$). Plant maturity influenced sap Ca concentration and Ca translocated increased as plant matured. These results provide evidence that flow rate differences may cause variability for pod Ca concentration in snap beans.

Pod Ca concentration among different snap bean genotypes varies (Quintana et al., 1996a), but physiological mechanisms that cause such variation are unclear. Soil Ca moves into the root apoplast until it reaches the endodermis, where it passes the Casparian strip via the symplast and enters the xylem (Baker and Hall, 1988). Ca transport continues upward in the transpiration stream and an exchange mechanism in which ascending Ca moves by ion substitution on biocolloids along the walls of the xylem vessels (Biddulph et al., 1961; Clarkson, 1984; Raven, 1986). Ca movement in the phloem is extremely low (Raven, 1977).

Several studies have shown the importance of pod transpiration on Ca accumulation in the developing snap bean pod (Mix and Marschner, 1976a, 1976b). Later work indicated that variation of pod Ca concentration among snap bean cultivars possibly was due to differences in the amount of Ca partitioned into the pods, probably influenced by pod transpiration (Grusak et al., 1996). We have shown that snap bean cultivars with greater pod stomatal densities do not necessarily have higher pod Ca concentrations (Quintana et al., 1996b), suggesting that variability of pod Ca concentration among genotypes could be due to differences in pod transpiration

associated with differences in stomatal size or transpiration rate, rather than stomatal density.

Roots function as absorbing surfaces through which water is absorbed by forces developed in the evaporating surfaces of the shoots (Kramer, 1983). However, when transpiration is low and soil is moist, warm, and well-aerated; healthy roots operate as osmometers that produce root pressure, which can cause guttation and bleeding from wounds and leaves (Kramer and Boyer, 1995). Permeability of plant membranes to water is much greater than to ions (Marschner, 1986); therefore, root pressure occurs because the concentration of solutes in the xylem is greater than in the external medium. These events cause a gradient in water potential that causes water to flow passively into the xylem (Flowers and Yeo, 1992).

Studies of xylemic exudation in tomato (*Lycopersicum esculentum* Mill.) (Ferrario et al., 1992), tobacco (*Nicotiana tabacum* L.) (Wallace et al., 1966), corn (*Zea mays* L.) (Canny and McCully, 1988; van de Geijn and Smeulders, 1981), and cucumber (*Cucumis sativus* L.) (Masuda and Gomi, 1982) have shown that rates and compositions of exudates are different among species. Root pressure provides sufficient Ca to nontranspiring tissue such as cabbage (*Brassica oleracea* L.) heads (Palzkill and Tibbitts, 1977; Tibbitts and Palzkill, 1979) and strawberry (*Fragaria ×ananassa* Duch.) fruits (Bradfield and Guttridge, 1979). Thus, some variability for snap bean pod Ca concentration could be caused by differences in root pressure among genotypes. Our objective was to examine the association between pod Ca concentration and flow rate exudate by effect of root pressure in snap bean genotypes that differ in pod Ca concentration.

Materials and Methods

EXPERIMENTAL DESIGN. Two intermittent aeroponic systems (Peterson and Krueger, 1988) were used simultaneously, and each consisted of 24 pots in which two plants, one each of 'Hystyle' and 'Labrador', were grown. In each replication, pots were divided randomly into three groups of eight pots each. Each group was

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randomly assigned a sampling time (flowering and 1 week and 2 weeks after flowering), and this resulted in eight pots (16 plants) per replication or 16 pots total (32 plants) being sampled at each stage. The experimental design was a split plot with sample time as the whole unit (whole plot) and cultivars the subunit (subplot) (Steel et al., 1996).

PLANT MATERIAL AND CULTURE. This research was conducted at the University of Wisconsin, Arlington Research Station, during Summer 1996 in a glass greenhouse. This greenhouse was equipped with evaporative cooling units to maintain temperatures during the summer. Two cultivars were selected from previous studies (Quintana et al., 1996a): 'Labrador', which exhibited low pod Ca concentration, and 'Hystyle', a high pod Ca concentration cultivar. In June, 1996, seeds of both cultivars were germinated under greenhouse (glass) conditions [28 °C, 60% relative humidity (RH) and no artificial light used] in plastic bags that contained a sheet of paper folded twice at the top to form a channel where four seeds were placed and watered. One of each cultivar of 10-d-old seedlings were transplanted into 20-L pots held in the greenhouse. Plants were suspended in a 2.5-cm-thick foam plastic lid that covered the pot. Air in the pot was constantly circulated and cooled with an air conditioning unit. Roots were misted with 10 mL of nutrient solution per pot at 15-min intervals. A modified nutrient solution composed of (in mg·L^{-1}): 57 N (as NO$_3$), 62 P, 191 K, 80 Ca, 24 Mg, 32 S, 2.7 Fe (as Fe DTPA, diethylenetriamine pentacetate), 0.5 Mn, 0.5 B, 0.3 Cu, 0.05 Zn, 0.01 Mo, and 0.01 Co was used (Peterson and Krueger, 1988). Nutrient solution pH was adjusted by adding 1 M NaOH as needed to approximate normal pH ranges (6.0 to 6.5). Root and shoot ambient temperatures were ≈24 and 28 °C, respectively, with RH maintained at ≈60%. No artificial light was used.

SAMPLING PROCEDURE. Snap bean plants were sampled at three growth stages: flowering (plants ≈30 d old), 1 week after flowering (pod formation), and 2 weeks after flowering (pods fully developed and ready to harvest) (LeBaron, 1974). Thus, 8 pots (16 plants) per replication or 16 pots (32 plants) total were sampled at each developmental stage. To be consistent with possible circadian rhythms (Taiz and Zeiger, 1991), all plants were excised during the afternoon (1700 HR) and left to exude over night (15 h).

DATA COLLECTION. Flow rate, Ca absorption, sap Ca concentration, pod Ca concentration, and foliage mass were measured. Each sampling consisted of 1) decapitation of the plant at the first node (≈10 cm above the ground); 2) covering the cut stem with a piece of preweighed, dry cotton; 3) wrapping the cotton with aluminum foil to prevent sap evaporation; 4) removing the cotton 15 h later and quickly reweighing it; and 5) storing cotton pieces in plastic bags at –2 °C to prevent sap losses and insure proper Ca extractions. Pods were collected and foliage (everything except root) mass (shoot fresh weight) was recorded only for the last sampling time (2 weeks after flowering). Pods were divided into two groups. One group consisted of only commercial size no. 4 (a premium grade, 8.3 to 9.4 mm in diameter), and the other group was a pooled sample of all pod sizes, ranging from 5.8 to 10.7 mm in diameter (Mullins and Straw, 1988).

CALCIUM DETERMINATIONS. Pods were oven-dried at 60 °C for 2 d and ground in a Wiley mill to pass a 10-mesh screen. A 0.05-g sample was weighed into a 10-mL glass beaker. Samples were dry-ashed in a muffle furnace at 450 °C for 5 h. When the samples cooled, 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 in a 50-mL volumetric flask. Filter paper was rinsed with two to three volumes of distilled–deionized water to make sure that all Ca was extracted from the ash. Ten milliliters of 0.2 N HCl that contained 10000 mg·L^{-1} lanthanum (as LaCl$_3$) was added to the extract to overcome chemical interferences (Macrae et al., 1993), and the total volume was brought to 50 mL with distilled–dionized water. Calcium was extracted from the cotton pads by leaching the cotton with distilled–deionized water several times. Then, 10 mL of 0.2 N HCl that contained 10000 mg·L^{-1} lanthanum was added to the extract and the volume was brought to 50 mL with distilled–deionized water. Ca concentration was determined by using an atomic absorption spectrophotometer (model Spectra AA-20; Varian Techtron Pty. Limited, Mulgrave Victoria, Australia).

TERMINOLOGY FOR FLOW RATE, CA ABSORBED AND CA CONCEN-TRATION IN POD AND SAP. To correct for the observed variability in shoot size among and within cultivars, flow rate was defined as the amount of xylemic exudate collected in one h divided by foliage mass (mL·h^{-1}·g^{-1}). Calcium absorbed was defined as mg of Ca collected in a period of one h divided by g of foliage mass (mg·h^{-1}·g^{-1}). The concentration of Ca in the sap (xylemic exudate) and pods was assumed not to be altered by variability in plant size, and therefore, units for these two variables were (mg·mL^{-1}) and (mg·g^{-1}), respectively.

STATISTICAL ANALYSES. Analyses of variance and correlations among pod Ca concentration, flow rate, Ca absorbed and sap Ca concentration were conducted only for the last developmental stage (2 weeks after flowering) because mature pods (commercial size no. 4) were present only at this stage. Cultivar means used for comparisons were calculated from all within replication plants of each cultivar. Experimental set up included two replications and each replication had eight separate measurements (plants). Statistical analyses were done by using SAS system software (SAS Institute, Cary, N.C.), and treatment means were separated by using LSD tests (Steel et al., 1996).

Table 1. Analysis of variance and cultivar means for flow rate, Ca absorbed, and Ca concentration of sap, no.4 pods (pod diameter = 8.3 to 9.4 mm), and total pods (a pooled sample of all pod sizes).[z,y]

Source	df	Flow rate	Ca absorbed	Ca concn		
				Sap	No. 4 pods	Total pods
Replication (R)	1	NS	*	NS	NS	NS
Pot (replication)	14	NS	NS	NS	NS	NS
Cultivar (C)	1	**	**	NS	*	**
C × R	1	NS	NS	NS	NS	NS
C means						
Hystyle		0.0092	0.0019	0.219	5.75	6.30
Labrador		0.0056	0.0011	0.232	3.71	4.29

[z]Analysis involves only data at harvest (2 weeks after flowering).
[y]Units for flow rate = mL·h^{-1}·g^{-1}), Ca absorbed = mg·h^{-1}·g^{-1}), Ca concentration of sap = mg·mL^{-1}) and pod Ca concentration = mg·g^{-1}).
[NS,*,**]Nonsignificant or significant at P = 0.05 or 0.01, respectively.

Table 2. Correlation coefficients for flow rate, Ca absorbed, sap Ca concentration, Ca concentration for pods size no. 4 (pod diameter = 8.3 to 9.4 mm) and total pods (a pooled sample of all pod sizes).[z][y]

			Ca concn		
	Flow rate	Ca absorbed	Sap	No. 4 pods	Total pods
Flow rate	---	0.90[**]	–0.27	0.55[**]	0.65[**]
Ca absorbed		---	–0.15	0.61[**]	0.57[**]
Sap			---	–0.28	–0.09
No. 4 pods				---	0.66[**]
Total pods					---

[z]Analysis involves only data at harvest (2 weeks after flowering).
[y]Units for flow rate = mL·h^{-1}·g^{-1}), Ca absorbed = mg·h^{-1}·g^{-1}), Ca concentration of sap = mg·mL^{-1}) and pod Ca concentration = mg·g^{-1}).
[**]Significant at $P = 0.01$.

Results and Discussion

FLOW RATE–POD CA CONCENTRATION RELATIONSHIP. Roots and shoots of snap bean cultivars showed no abnormalities during development. Differences among cultivars ($P < 0.01$) were observed for flow rate and pod Ca concentration (Table 1). Flow rate of 'Hystyle' (0.0092 mL·h^{-1}·g^{-1} foliage mass) was 1.6 times greater than of 'Labrador' (0.0056 mL·h^{-1}·g^{-1} foliage mass) (Table 1). Likewise, with 'Hystyle' Ca concentration for no. 4 pods (5.75 mg·g^{-1}) and total pods (pooled sample of all sizes) (6.3 mg·g^{-1}) were 1.6 and 1.5 times greater than those in 'Labrador' (Table 1). These pod Ca concentration values are consistent with those found in our previous field studies and ratios detected in hydroponic studies. Flow rate correlated positively with pod Ca concentration in no. 4 pods ($R = 0.55$) and total pods ($R = 0.65$) (Table 2).

Even though we did not determine the root system size in the two cultivars, we observed no apparent differences in root growth. The foliage size did vary among the two cultivars as well as among various plants within a given replication. Thus, in order to determine if exudate flow was contributing to the Ca accumulation in the plant; the data on flow rate was normalized with respect to the foliage fresh weight. Mean ± sd values for fresh foliage weight were 78.1 ± 7.4 g and 139.4 ± 0.3 g for 'Hystle' and 'Labrador', respectively. In the two tail t test, $P = 0.054$.

CALCIUM ABSORBED. Total Ca absorbed in 'Hystyle' (0.0019 mg·h^{-1}·g^{-1} space foliage mass) was ≈60% higher than that found in 'Labrador' (0.0011 mg·h^{-1}·g^{-1} foliage mass) (Table 1). Statistical analysis showed a positive correlation of $R = 0.90$ between flow rate and Ca absorbed (Table 2). Calcium absorption was associated positively to Ca concentration of no. 4 pods (R = 0.61) and total pods (R = 0.57) (Table 2). This implies that increased flow into the plant during the night was able to bring more Ca to the plant including the fruits. These results are also consistent with the data from previous studies demonstrating that some crop plants appear to deliver Ca to transpiring leaves during the day and to low-transpiring organs at night (Clarkson, 1984; Tibbitts and Palzkill, 1979).

CALCIUM CONCENTRATION IN XYLEM SAP. Average Ca concentration in the exudate (0.226 mg·mL^{-1}) was 2.8-fold that found in the nutrient solution (0.080 mg·mL^{-1}). Absorption of ions by the roots raises the internal solute concentration, thereby resulting in a gradient of water toward the roots, which tends to balance the osmotic pressure difference between the xylem and external medium. Thus, these results are in agreement with the osmotic principle that root pressure is the predominant cause of xylem exudation in plants (Mengel and Kirkby, 1982). Cultivar differences for sap Ca concentration were not detected (Table 1), and this suggests that variability of pod Ca concentration among cultivars is caused mainly by quantitative differences in flow rate, rather than qualita-

tive differences of sap. However, in this study sap measurements were done at 15-h intervals, and intermediate time values are unknown. Therefore, it is possible that variability for sap Ca concentration among cultivars could be detected by sampling more frequently.

Calcium concentration in the sap was independent of flow rate ($R = -0.27$) and total Ca absorbed ($R = -0.15$) (Table 2). Low-negative values were observed for correlations between Ca concentration in sap and no. 4 pods ($R = -0.28$), and Ca concentration in sap and total pods ($R = -0.09$). In this study, sap Ca concentration had little effect on pod Ca concentration in snap beans.

The amount of exudate collected in 15 h did not change significantly as plants matured ($P > 0.05$). The amount of Ca absorbed and xylem Ca transported upwards in the sap increased with plant maturity (Table 3), and this provided more evidence for the strong association between Ca absorbed and flow rate (Table 2). This observation makes sense because the Ca requirements of a plant increase with the constant production of Ca sinks during plant development and fruit expansion. During growth, xylem branches ascend into stems and developing leaves and fruits. Thus, the terminal branches are close to the dividing and expanding tissues, and this insures apoplastic continuity (Hanger, 1979). Cation exchange sites within the new walls of the growing tissues are continuous with those in the xylem, and as tissues grow, they provide a sink for Ca in the xylem exchange column and Ca migrates toward them (Clarkson, 1984).

Sap Ca concentration differed among developmental stages (Table 3). The oldest plants had the greatest sap Ca concentrations, and this suggested that plant maturity influences overnight Ca concentration in the xylem. Perhaps as plants age, sap Ca concentrations in the xylem increase at night due to greater demand for Ca by newly synthesized tissue (Clarkson, 1984). It also may be possible that in snap beans, sap Ca concentration follows a daily cycle similar to tomatoes (Ferrario et al., 1992) or changes with environmental conditions (Canny and McCully, 1988). Despite

Table 3. Mean LSD comparisons for Ca absorbed and sap Ca concentration among three developmental stages for snap bean plants grown under aeroponic conditions.[z]

Stage	Time (d)	Ca absorbed (mg·h^{-1})	Sap Ca concn (mg·mL^{-1})
Flowering	30	0.114 ± 0.039	0.196 ± 0.046
Pod filling	37	0.121 ± 0.049	0.179 ± 0.034
Harvest	44	0.153 ± 0.072	0.225 ± 0.050
LSD ($P< 0.05$)		0.053	0.022

[z]Each value is the mean ± sd of measurements of all plants within each stage.

changes in sap Ca concentration with circadian rhythms or environmental variation, it can be proposed that a growing bean plant satisfies its Ca requirements to low-transpiring organs during the night period via continuous accretions in the amount and concentration of sap transported upward via flow rate by effect of root pressure.

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