Changes in Sugar Concentrations of Seed and Pod Tissue During Development in Snap and Dry Beans (*Phaseolus vulgaris* L.)

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Abstract. **Sugars, including glucose, fructose, and sucrose, contribute significantly to the flavor and consumer acceptance of snap beans (*Phaseolus vulgaris* L.). Sugar accumulation and changes in sugar profiles during snap bean development contribute to overall assessments of quality for breeding lines and cultivars. Developing fruit from a diverse group of four snap bean cultivars containing Andean germplasm and one Mesoamerican dry bean cultivar were sampled at 5-day intervals from 10 to 30 days after flowering over 2 years. Glucose, fructose, and sucrose in pod and seed tissue was quantified using high-performance liquid chromatography. Percent seed mass relative to pod mass increased with days after flowering, but the rate of increase was heterogeneous among cultivars. Significant differences in sugar accumulation patterns of mono- and disaccharides were observed with time of development and between pods and seeds. Glucose and fructose decreased rapidly in pods and seeds with time after flowering. In contrast, sucrose concentration increased in pod tissue but remained constant in seeds of the snap bean cultivars with time after flowering. The patterns of changes in pod and seed sugar concentrations with time after flowering were similar among all snap bean cultivars. In contrast to the snap beans, seed sucrose increased with time after flowering in the Mesoamerican dry bean cultivar Puebla 152. No year by day after flowering interactions were observed for sugar accumulation patterns or sugar concentrations. Younger snap beans had the highest sweetness index based on observed sugar concentrations, percent seed mass, and perception of relative sweetness by the human palate. Although mean sweetness varied between cultivars, the rate of decrease in sweetness with time was the same for all five cultivars. These findings indicate that variation for sweetness exists in snap beans and can be exploited by breeding to develop cultivars with a potentially more desirable, sweet flavor.**

The USDA Food and Nutrition Information Center promotes the increased consumption of fruits and vegetables for better health (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015). Breeding vegetables for enhanced nutrient content and other traits requires estimation of genetic variance among genotypes based on repeatable measures at comparable stages of maturity. Cereals and pulses are generally harvested at physiological maturity, have a high starch content, low moisture content (6% to 14%), and low rates of respiration. Thus, meaningful evaluations of differences among genotypes or treatments can often be made over extended periods of time if samples are stored under similar conditions (Górecki et al., 2001). The same is often true for root and bulb vegetables, such as carrots (*Daucus carota*) and onions (*Allium cepa*), which are harvested at or near physiological maturity and can be stored for extended periods of time with little change in nutrient content. In contrast, many other vegetables are harvested when they are immature and have high sugar and moisture contents and high rates of respiration. Thus, chemical constituents can change quickly, often in a matter of hours after harvest.

Culinary quality of many vegetables is often inversely related to size and maturity, especially those in which the vegetable is an immature seed or fruit. Examples include snap beans, peas, sweet corn, and cucumbers. In general, smaller, immature tissues command greater consumer acceptance due to higher sugar content and reduced fiber (Bianco and Pratt, 1977; McCollum et al., 1988; Robinson et al., 1963). For these vegetables, one needs to establish methods of phenotypic evaluation that allow breeders to make meaningful selections despite differences between genotypes in rates of development and size that may be correlated with traits of interest.

Modern snap beans are bred for small seed size compared with pod tissue, and one proxy for maturity used by the processing industry is seed length. This measure is often not useful in comparisons among cultivars as relative seed elongation rates vary widely between cultivars (Carr and Skene, 1961). Pod diameter, or sieve size, has also been used as a proxy for maturity, with the advantage that it reflects commercial marketability. Nevertheless, variation in rates of pod development as well as differences in pod shape (round, oblate, or flat) may confound comparisons among genotypes (VandenLangenberg et al., 2012). Days after flowering is an additional measure of maturity that has been used for comparisons among genotypes, although variation in rates of maturity could result in biased comparisons.

Fruits of *Phaseolus vulgaris* contain a single carpel that develops from the ovary, remnants of additional floral parts, and seeds (Jones and Luchsinger, 1986). In this article, the developing maternal tissues, with the exception of maternal tissues such as the testa that adhere tightly to the seeds, are collectively referred to as the pod. Fertilization occurs on the day of anthesis (Walbot et al., 1972). Pod growth begins ≈2 d later (Carr and Skene, 1961) and is supported by sucrose imported from leaves and sucrose supplied by photosynthetic pod tissues (Carr and Skene, 1961; Lucas et al., 1976; Patrick and McDonald, 1980; Walbot, et al., 1972). Dry matter content of pods peaks 16 to 24 d after anthesis, depending on the cultivar (Carr and Skene, 1961; Sung et al., 1994; Walbot et al., 1972). The onset of embryo growth is delayed relative to pod growth. Early embryo growth is supported by the endosperm, which is consumed by the end of the cotyledon

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Table 1. Origin, plant, pod, and seed characteristics of germplasm accessions from the U.S. Department of Agriculture *Phaseolus vulgaris* collection used in evaluation of pod and seed sugar concentrations.

PI No.	Accession name	Released by (yr)[z]	Plant growth habit	Pod length (cm)[y]	Seed wt (g per 100)	Market class
PI 550219	Champagne	Billie Hepler Seed Co., USA (1952)	Indeterminate climbing	25	42.2	Snap, dry pole
PI 549903	Goldcrop	U.S. Department of Agriculture, USA (1974)	Determinate bush	13	25.8, 32.0	Wax
PI 550288	Hystyle	Harris Moran Seed Co., USA (1985)	Determinate bush	13	32.5	Snap
PI 549577	Morse's Pole No. 191	Ferry-Morse Seed Co., USA (1936)	Indeterminate climbing	18	50.0	Snap
PI 661928	Puebla 152	Heirloom landrace, Mexico	Indeterminate climbing	10	30.0	Black bean

[z]Billy Hepler Seed Company, 1952; Ceccatto et al., 1998; Wehner, n.d.
[y]Mean pod length in centimeters of 10 selected pods at the completion of the pod-filling stage.

stage, ≈18 d after fertilization (Brown, 1917; Geiger et al., 1989; Walbot et al., 1972). The carbohydrates used for seed growth come primarily from sucrose delivered by the maternal seedcoat to the apoplastic space and taken up by the cotyledons (Patrick and McDonald, 1980). Short-term $^{14}CO_2$ labeling studies showed that 80% of the soluble carbohydrate that accumulated in cotyledons was sucrose, with less than 5% each of glucose and fructose (Patrick and McDonald, 1980). Significant accumulation of seed dry weight begins ≈8–12 d after fertilization, stops briefly around 20–24 d after fertilization and then resumes until ≈28–32 d after fertilization (Carr and Skene, 1961; Geiger et al., 1989; Loewenberg, 1955; Sung et al., 1994; Walbot et al., 1972).

Although all plant tissues contain sugars, developmental differences can affect sugar concentrations and relative amounts of individual sugars found in vegetables and specific vegetable tissues (Hounsome et al., 2008; Lee et al., 1970). Regardless of the tissue type, sugar concentrations are cultivar dependent and highly variable (Hounsome et al., 2008; Lee et al., 1970). In snap beans, studies characterizing simple sugar concentrations in fruits have often focused on a single cultivar at a single developmental stage (Lee et al., 1970; Muir et al., 2009; Sánchez-Mata et al., 2002). For example, fruit of cv. Tender Green contained 16.5% reducing sugars and 3.45% sucrose on a dry weight basis at harvest (Williams et al., 1948). Significant differences in sugar accumulation patterns and quantity were observed between a dry bean cultivar and five snap bean cultivars in which the fruit were sampled at sieve size 4 (Vandenlangenberg et al., 2012). Fruit at sieve size 4 have a maximum diameter between 5.7 mm and 8.3 mm and are commonly commercialized as fresh or frozen snap beans.

In a few cases, sugar contents in *P. vulgaris* fruit or fruit tissues have been sampled at multiple times of development. Reducing sugars, primarily fructose and glucose, in the snap bean cultivar Burpee's Stringless Green Pod, decreased in pods from 2% to 1% and in seeds from 0.25% to trace amounts of fruit fresh weight from 15 to 40 d after flowering (Culpepper, 1936). In contrast, the content of nonreducing sugars, primarily sucrose, in pods was relatively constant at 0.5% but decreased dramatically from 3.2% to 1% in seeds from 15 to 40 d after flowering (Culpepper, 1936). In a recent study that compared sugar contents in fruits of a dry bean and five snap bean cultivars, fructose and glucose content in the snap bean cultivars decreased from a mean of 75 and 40 $mg \cdot g^{-1}$ dry weight to less than 40 and 25 $mg \cdot g^{-1}$ dry weight, respectively, as pod diameter increased from 5.8 to 10.7 mm (VandenLangenberg et al., 2012). In contrast, sucrose concentration increased with increasing diameter from near 0 to ≈10 $mg \cdot g^{-1}$ dry weight in the five snap bean cultivars (VandenLangenberg et al., 2012). At pod diameters between 5.8 and 9.7 mm, fructose and glucose concentrations were 10 to 20 times higher than sucrose concentrations in all snap bean cultivars. Sucrose concentrations were higher relative to glucose and fructose concentrations when the diameter increased to 10.7 mm. No distinction was made in sugar concentrations of seed vs pod tissue.

The objectives of this study were 3-fold: 1) measure glucose, fructose, and sucrose concentrations in a dry bean and four snap bean cultivars using days after flowering as a measure of pod development; 2) monitor changes in sugar content in developing seeds compared with pod tissue during fruit development; and 3) determine if days after flowering is a useful measure of maturity to use when making comparisons between genotypes.

Materials and Methods

Plant material. Four edible-podded beans representative of diverse market classes and a Mesoamerican dry bean landrace were included in the study (Table 1). The edible podded beans included cultivars that have determinate and indeterminate growth habits, and differ in pod length and seed weight. Morphological traits, including the size of seeds, pods, and leaves and leaf morphology, indicate that these four cultivars contain Andean germplasm (Singh et al., 1991). The modern large sieve, small seed size processing snap bean cultivar Hystyle, was selected to contrast with earlier home garden wax and pole bean cultivars. Two of the cultivars included in the present study, Hystyle and Puebla 152, were included in a previous study of sugar concentrations in *P. vulgaris* fruit (VandenLangenberg et al., 2012).

Plant culture and sampling. The five cultivars were planted in 2016 and 2017 at the West Madison Agricultural Research Station (WMARS), Verona, WI 53593 using standard cultural practices for snap beans (Binning et al., 1998). The soil type at WMARS is a Plano silt loam. Fields were treated with Duel II Magnum herbicide, Treflan MTR herbicide, and Lorsban-4E insecticide 2 d before planting to stunt the early development of pests and weeds. In 2016, air temperatures were within normal ranges during the growing season of June to October. In contrast, record high rainfall occurred in 2016 with fewer but more intense rainstorms than average. Temperatures in June and Oct. 2017 were warmer than normal, with October temperatures hitting record highs. Rainfall in 2017 was higher than in 2016 between 19 June and 19 Aug., after which rainfall was below normal (Wisconsin State Climatology Office, 2011). Manual irrigation was used sparingly in both years on an as needed basis.

Fifty seeds of each of the five selected cultivars were hand-planted in 7.6-M rows and repeated in a second repetition in each year using a randomized complete block design where each block was a planting date. Blocks were planted 2 weeks apart to stagger harvesting dates and facilitate sampling. In 2016, blocks were planted on 17 June and 1 July. In 2017, blocks were planted on 31 May and 12 June. As flowers opened, they were labeled using waterproof Tyvek tags and wire or string. In some cases, the day a flower opened was estimated based on visual appearance of the color and turgidity of the corolla. After the flowers set fruit, five fruit of each cultivar were randomly harvested and pooled over replications for high-performance liquid chromatography (HPLC) analysis at 10, 15, 20, 25, and 30 d after flowering. Harvested fruit were immediately photographed, bagged, and placed in a cooler until they were placed in a –80 °C freezer, typically within 1 h of harvest. Flower abortion rates were higher than expected in both replications over both years. Because of erratic flowering in 2017, fewer than five fruit of 'Champagne' and 'Morse's Pole No. 191' were harvested for some sampling periods. These cultivars were planted the following winter (Dec. 2017) in the Walnut Street Greenhouse, Madison, WI, and fruit harvested as needed to provide complete data for each sampling date after flowering. Analysis of normal quantile plots of residuals did not reveal any observed pattern of differences in field and greenhouse sugar data; thus, the greenhouse data for 'Champagne' and 'Morse's Pole No. 191' were combined with the 2017 field data.

Soluble-sugar extraction. Frozen whole fruit samples were lyophilized using a VirTis Freezemobile 25XL (SP Industries, Warminster, PA). Each dried sample was partitioned into pod and seed subsamples by carefully hand-separating seeds from pods. Partitioned samples were weighed and ground to a fine powder by shaking with 4.5-mm-diameter metal beads in a modified electric paint

Table 2. Mean squares and year means for pod and seed sugars (mg·g^{-1} dry weight) for four edible podded and one Mesoamerican dry bean cultivar evaluated over 2 years at 10, 15, 20, 25, and 30 d after flowering (DAF).[z]

		Mean squares for tissues and sugar concentrations					
		Pod sugars (mg·g^{-1})			Seed sugars (mg·g^{-1})		
Source	df	Sucrose	Glucose	Fructose	Sucrose	Glucose	Fructose
Year[y]	1	39.2^{ns}	249.0^{ns}	446.5*	127.5^{ns}	137.8*	407.9**
Cultivar	4	7.9^{ns}	901.4***	375.7*	12.2***	55.6*	199.9**
Cultivar × year	4	56.8^{ns}	383.0*	175.9^{ns}	205.1*	54.5^{ns}	124.3*
DAF	3[y]	1142.2***	2523.0***	6023.8***	467.5**	372.4***	2060.2***
DAF × year	3[y]	75.3*	99.5^{ns}	248.9^{ns}	23.7^{ns}	40.2^{ns}	162.6*
DAF × cultivar	3[y]	23.4^{ns}	192.9^{ns}	224.7^{ns}	85.5^{ns}	15.7^{ns}	42.7^{ns}
Pooled error	15	22.8	101.1	96.9	61.4	19.0	33.8
				Means over years			
2016		7.96	57.3	94.0	36.8	12.0	29.7
2017–18[x]		4.00	47.6	80.7	29.7	19.4	42.5

[z]Edible podded snap bean cultivars included Champagne, Goldcrop, Hystyle, and Morse's Pole No. 191, the dry bean was a Mesoamerican black bean landrace, Puebla 152. Puebla was evaluated only to 25 d after flowering.
[y]Lost df because cultivar Puebla 152 was evaluated only up to day 25.
[x]Due to lost field data for some sampling dates in Summer 2017, the second year (2018) data for cultivars Champagne and Morse's Pole No. 191 were collected in a greenhouse planting during Winter 2017–18.
^{ns}, *, **, ***Nonsignificant or significant at 0.10, 0.05, or 0.01, respectively.

shaker. Powdered samples (0.10 g) were extracted twice by incubating with 4.0 mL of 80% ethanol and shaking at 250 rpm for 24 h in a G24 Environmental Incubator Shaker (New Brunswick Scientific Co., Inc., Edison, NJ) at 60 °C. Extracts were centrifuged at 13,000 g_n at 20 °C for 10 min to pellet solid material. Supernatants from both extractions were pooled together and brought to 10.0 mL by adding 80% ethanol. Supernatant extract samples (1.5 mL) were dried under vacuum without heating using an Eppendorf Vacufuge (Eppendorf North America, Hauppauge, NY), resuspended in 1.0 mL ultrapure water, and filtered through a Sep-Pak C18 cartridge (Waters Associated, Inc., Milford, MA) that was washed with 5.0 mL methanol followed by 5.0 mL ultrapure water and 5.0 mL air. Lastly, samples were filtered through a 0.2-µm Millipore membrane (Corning Inc., Corning, NY) and stored at –20 °C until used for HPLC analysis.

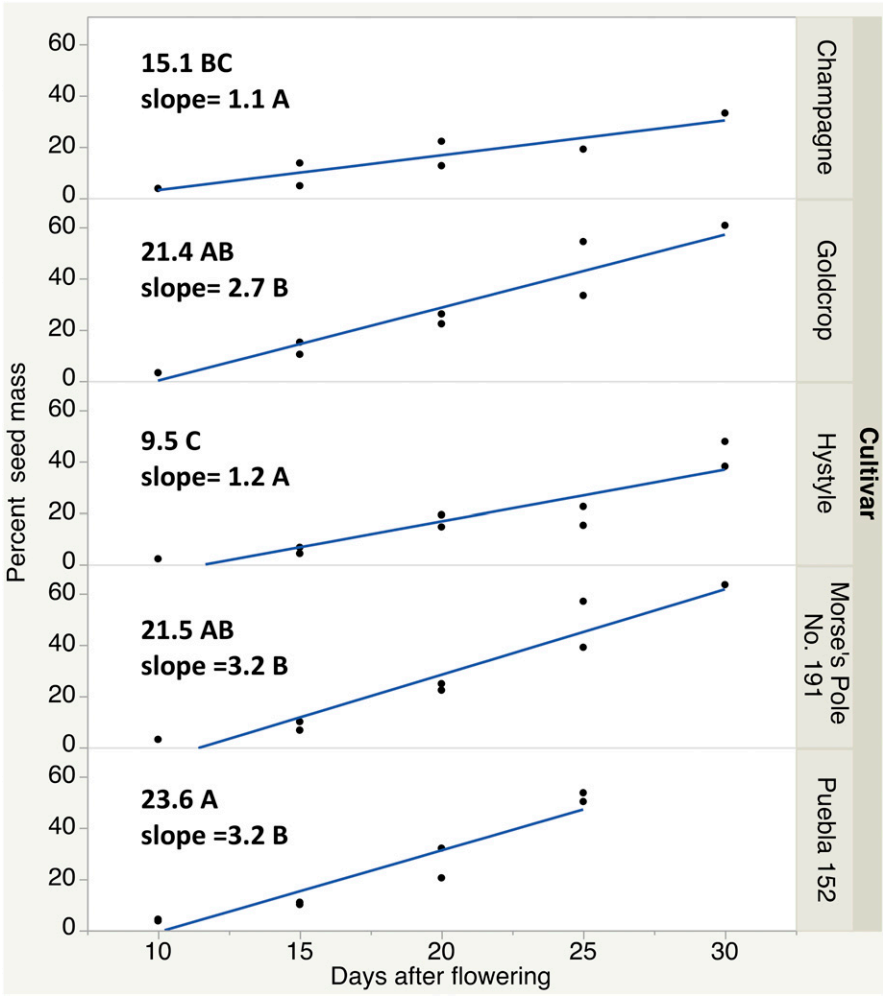

Fig. 1. Percent seed mass of *P. vugaris* fruit sampled over five day intervals after flowering for four edible podded cultivars (Champagne, Goldcrop, Hystyle and Morse's Pole No. 191) and one Mesoamerican black seeded dry bean landrace (Puebla 152). Puebla 152 was sampled only up to 25 days after flowering. Numerical values are mean percent seed mass from 10 to 25 days after flowering. Means not connected by same letter are significantly different at a level of significance of $P<0.05$ (t-test). Linear regression coefficients for increasing percent seed mass over days after flowering were all significant ($P< 0.05$). Slopes not connected by the same letter are significantly different ($P<0.05$) based on testing heterogeneity among all pairs of cultivars.

Thawed sugar extracts (10 µL) were analyzed with a Shimadzu Prominence HPLC system (Shimadzu Scientific Instruments, Columbia, MD) using a 300 × 7.8 mm Rezex ROA-organic acid column (Phenomenex, Torrance, CA) with 0.0041% HPLC grade formic acid (pH 3.30 ± 0.02) (Sigma-Aldrich, St. Louis, MO) in water as the mobile phase at a flow rate of 0.59 mL·min^{-1} and a Shimadzu refractive index detector. Glucose, fructose, and sucrose were identified and quantified based on characteristic retention times and HPLC peak areas were converted to sugar concentration using a calibration curve produced from sugar standards that were included with each HPLC sample set. Glucose, fructose, and sucrose were easily separated from other compounds in the HPLC chromatogram.

Statistical analysis. Data were analyzed independently for glucose, fructose, and sucrose concentrations in seed and pod tissue using the statistical package JMP Pro 13 (JMP SAS Institute Inc., Cary, NC). Dried seed mass as a percentage of total dried fruit mass (percent seed mass) and sugar concentrations were subjected to Box-Cox transformations to improve normality. Inspection of scatter plots of transformed compared with nontransformed data for percent seed mass and sugar concentrations indicated that transformations resulted in improved normality only for seed

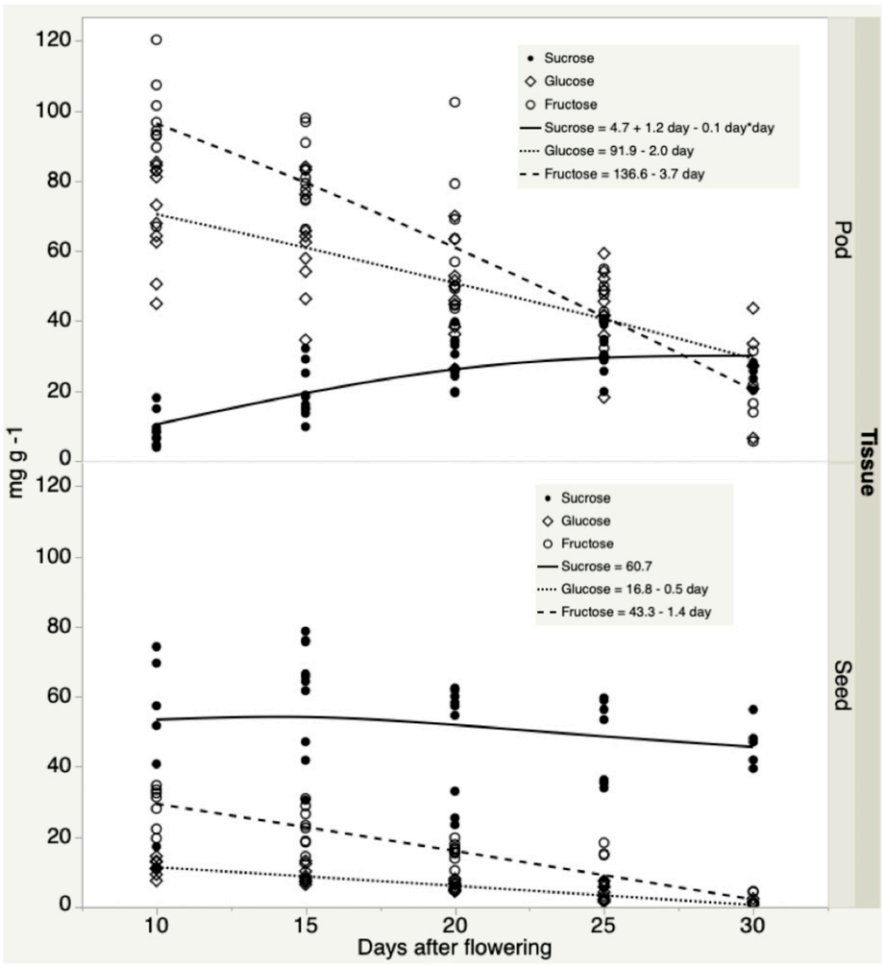

Fig. 2. Changes in dry weight sucrose, glucose, and fructose sugar concentrations (mg·g^{-1}) (Box-Cox transformed) in bean pod and seed tissue from a sample of five *Phaseolus vulgaris* cultivars sampled over 5-d intervals beginning 10 d after flowering. Regression equations reflect only significant (0.05 level) parameter estimates.

mass data below 10% and sugar concentrations lower than 10 mg·g^{-1} dry weight. Differences in mean percent seed mass relative to pod mass from 10 to 25 d after flowering were assessed using a *t* test. Heterogeniety among cultivars in rate of change in seed mass relative to pod mass was evaluated using linear regression. Differences in coefficients associated with change in percent seed mass over time were evaluated by testing for heterogeneity among all pairs of cultivars.

Analysis of variance (ANOVA) was performed for glucose, fructose, and sucrose development over time in pods and seeds to identify significant differences between cultivars, environments, and time after flowing.

A relative sweetness score was calculated by converting the contribution that each sugar makes to detectable sweetness into a single standardized unit. Glucose is perceived to be 74% as sweet as sucrose, whereas fructose is 117% as sweet as sucrose (Joesten et al., 2007). Therefore, the sweetness modifiers for sucrose (1.0), glucose (0.74), and fructose (1.17) were applied to the concentrations of sucrose, glucose, and fructose found within the whole fruit. The calculations for sweetness score were made using the appropriate coefficients for each sugar as follows:

$$Sweetness\ score = \frac{total\ mg\ sugar}{g\ dry\ wt.\ fruit} * \frac{1\ g}{1000\ mg} * \frac{1}{\frac{Mol.\ Weight\ sugar}{Sweetness\ Modifier\ for\ sugar}} * \frac{}{1}$$

A relative sweetness index for *P. vulgaris* fruit with units of sucrose equivalents per gram dry weight was calculated as the sum of the sweetness scores (SS) for the three sugars.

$$Sweetness\ Index = SS\ Suc + SS\ Glc + SS\ Fru$$

Mean sweetness index of each cultivar over days after flowering and linear regression coefficients for changes over time were calculated. Heterogeniety among linear regression coefficients associated with changes in the sweetness index over days after flowering was assessed by evaluating heterogeneity among all pairs of cultivars.

Results and Discussion

Sugar contents in pods and seeds varied with time after flowering and between tissues. ANOVAs for glucose, fructose, and sucrose in pod and seed tissues revealed that the largest source of variation was "days after flowering," with values ranging from 2 to more than 100 times larger than the mean squares values associated with year, cultivar, or year-by-cultivar interactions (Table 2). Sugar concentrations were generally higher in 2016 than 2017–18, with the exception of seed glucose and fructose. This may be due to generally more favorable field growing conditions in 2016 compared with 2017.

Significant (0.05 level) cultivar by year interactions were observed for pod glucose and seed sucrose and fructose concentrations; however, day-by-cultivar interactions were all nonsignificant, indicating that the ranking of the pod and seed sugar concentrations of the cultivars was consistent over sampling dates after flowering (Table 2). Residual normal quantile plots did not indicate a difference in the pattern of field compared with greenhouse data for missing sampling dates (data not shown). This suggests that the inclusion of greenhouse-grown samples to make up for partial missing 2017 field samples for 'Champagne' and 'Morse's Pole No. 191' did not bias the pod and seed sugar concentration data. This observation is consistent with a prior study of five snap bean cultivars and one dry bean cultivar in which significant genotype-by-year interactions were not observed for fruit sucrose, glucose, and fructose concentrations over years (VandenLlangenberg et al., 2012).

'Hystyle', a modern large-sieve processing snap bean cultivar released in 1985, had the lowest percent seed mass when averaged over the 10 to 25 d after flowering time period (Fig. 1). Two of the remaining edible podded cultivars, Goldcrop and Morse's Pole No. 191, were developed earlier and had larger percent seed mass compared with Hystyle, which is consistent with recent breeding objectives and consumer preferences to minimize seed size relative to pod size. A relatively high mean percent seed mass, 21.8% when averaged 5 to 30 d after flowering, was observed for 'Burpee's Stringless Green Pod', a cultivar released in 1894 (Culpepper, 1936). The dry bean cultivar Puebla 152 had the highest mean percent seed mass, 23.6%, of the five used in this study, which is consistent with breeding objectives in dry beans to increase yield with either more or larger seeds.

Linear regression coefficients for percent seed mass over days after flowering were all significantly different from zero, but heterogeneous among cultivars. The rate of percent seed mass increase over days after flowering was slowest in 'Hystyle' and 'Champagne' compared with the other cultivars. The rate of percent seed mass increase in 'Burpee's

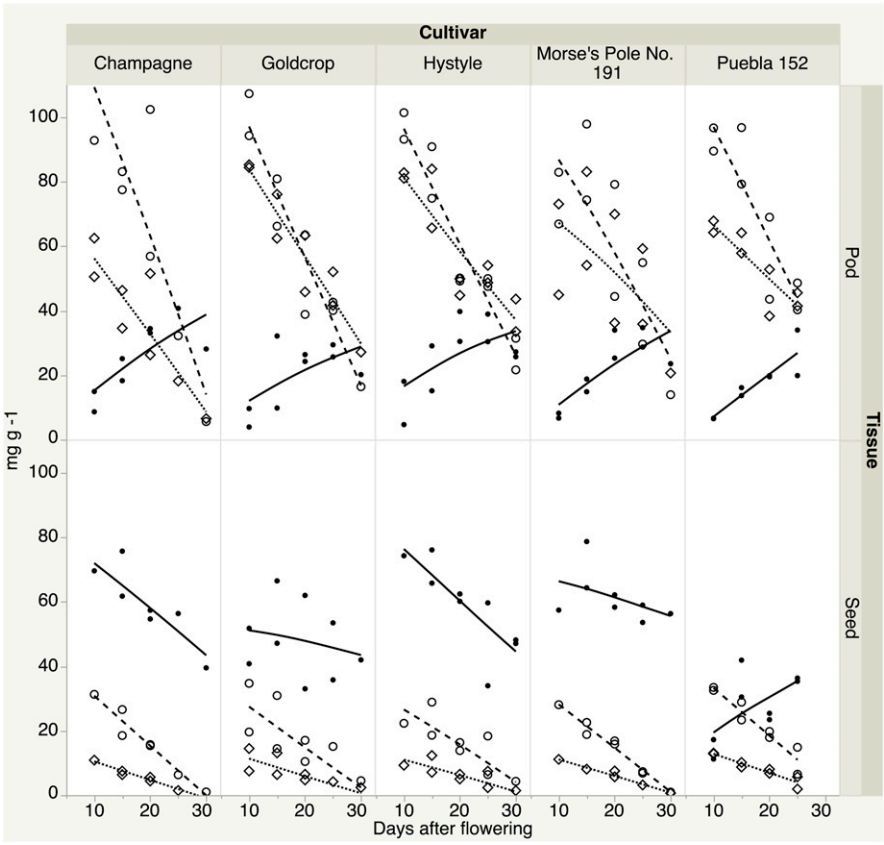

Fig. 3. Changes in dry weight sucrose, glucose, and fructose sugar concentrations (mg·g^{-1} dry weight) (transformed data) in pod and seed tissues of four Andean edible podded bean cultivars (Champagne, Goldcrop, Hystyle, and Morse's Pole No. 191) and a Mesoamerican black seeded dry bean landrace, Puebla 152, sampled over 5-d intervals beginning 10 d after flowering.

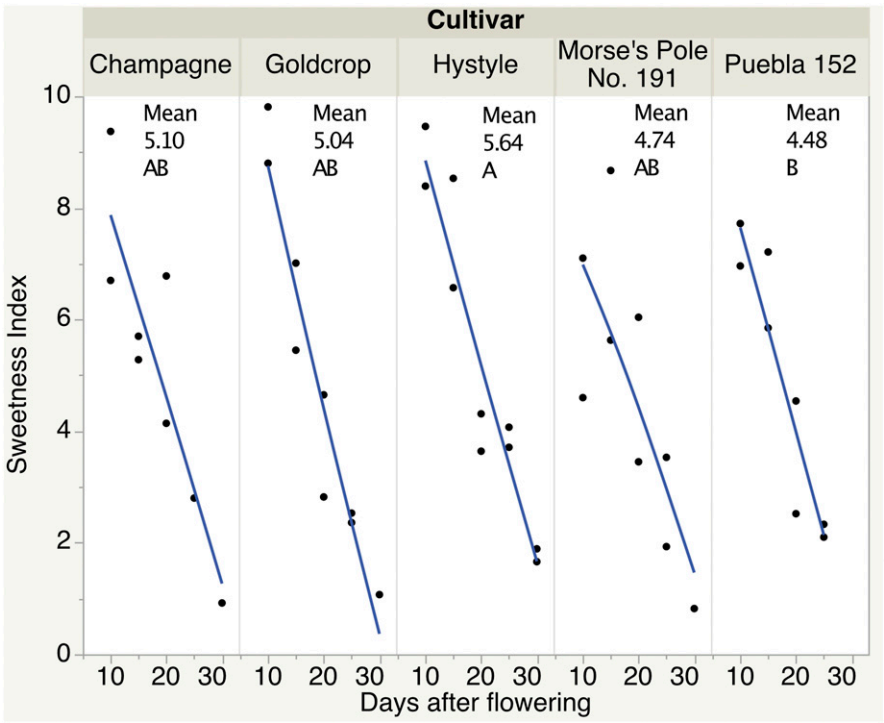

Fig. 4. Relative sweetness index per gram dry weight of combined seed and pod tissue weighted by relative detectable sweetness to the human palate of sucrose (1.00), fructose (1.17), and glucose (0.74) sugar concentrations sampled over 5-d intervals from 0 to 30 d after flowering. Overall cultivars the mean linear regression equation for sweetness index = 11.4–0.34* d after flowering. 'Puebla 152' was sampled only up to 25 d after flowering. Slopes for all cultivars were not significantly different from each other.

Stringless Green Pod' was 2.9% per day (Culpepper, 1936), which is similar to the rates measured in the present study in 'Goldcrop', 'Morse's Pole No. 191', and 'Puebla 152', 2.7%, 3.2%, and 3.2% per day, respectively. These data indicate that the rate of increase in dry matter accumulation in seeds relative to pods is variable among cultivars and confirms how it was possible to develop a modern snap bean cultivar such as Hystyle with small seed relative to pod tissue. Moreover, this result suggests that the rates of seed and pod development are under separate genetic control.

In general, an inverse relationship between glucose and fructose concentrations compared with sucrose concentration was observed over all cultivars and years in pods (Fig. 2). Pod glucose and fructose concentrations decreased with days after flowering from ≈75 and 100 mg·g^{-1} dry weight to 30 and 20 mg·g^{-1} dry weight, respectively, between 10 and 30 d after flowering. There was a curvilinear increase in pod sucrose concentration from ≈10 to 25 mg·g^{-1} from 10 to 30 d after flowering. In contrast, sucrose concentration in seeds remained relatively constant at ≈55 mg·g^{-1} dry weight. Concentrations of seed fructose and glucose decreased from 30 mg·g^{-1} dry weight and 10 mg·g^{-1} dry weight, respectively, at 10 d after flowering, to nearly 0 mg·g^{-1} dry weight at 30 d after flowering. Results in the present study are consistent with a prior study of five snap and one dry bean cultivars, where fructose and glucose in whole fruits decreased from ≈75 to 25 mg·g^{-1} dry weight as sieve size increased from 1 to 5 (Vandenlangenberg et al., 2012).

Changes in pod glucose, fructose, and sucrose concentrations with days after flowering were similar across all cultivars, and linear regression coefficients for rate of change in sugar content were significantly different from zero (data not shown) (Fig. 3). Changes in seed glucose and fructose concentrations with days after flowering were similar for all cultivars, and linear regression coefficients were significantly different from zero (data not shown) (Fig. 3). Reductions in pod glucose and fructose and an increase in pod sucrose with time were also observed in fresh pod tissue of cultivar Burpee's Stingless Green Pod (Culpepper, 1936). In addition, the increase in pod sucrose concentration and reduction in pod glucose and fructose concentrations is consistent with the changes in whole fruit sugar concentrations observed in five snap bean cultivars (including Hystyle) and the dry bean cultivar Puebla 152 (Vandenlangenberg et al., 2012). In all edible podded beans, the seed sucrose concentration decreased with days after flowering. In contrast, a significant linear increase in seed sucrose concentrations with increasing days after flowering was observed in the Mesoamerican dry bean cultivar Puebla 152. Sucrose is the primary soluble sugar in seeds (Bailly et al., 2001; McPhee et al., 2002). The reduction in seed sucrose concentration in days after flowering may be due to sucrose being converted enzymatically into other,

more complex seed storage sugars or starch as seeds approach physiological maturity.

Sucrose transported into the bean pod through the phloem is the primary source of carbon and metabolic energy during pod development (Lucas et al., 1976; Weber et al., 1997). During the early stages of pod development, activity of acid invertase increases and reaches a peak at the time of maximum dry weight accumulation (Sturm, 1999). Invertase hydrolyzes sucrose into fructose and glucose, and it seems likely that the high concentrations of glucose and fructose relative to sucrose observed during this early period of development (Figs. 2 and 3) reflect the activity of acid invertase. As snap bean pods reach later stages of growth, beyond 10 to 14 d postanthesis, acid invertase activity rapidly decreases and sucrose synthase activity increases (Sung et al., 1994). In seeds, however, acid invertase activity was low and sucrose synthase was the predominant enzyme used to cleave sucrose and generate substrates for growth, respiration, and starch accumulation (Sung et al., 1994).

Soluble sugar concentrations, including glucose, fructose, and sucrose, can improve the consumer perception of taste and flavor of snap and dry beans either directly or through enhancement of other organic compounds (Auerswald et al., 1999). In general, if sweetness is perceived in a vegetable, even mildly, it may be enough to encourage consumption (Dinehart et al., 2006). Sensory panelists who sampled dry beans and edamame (*Glycine max* L. Merr.) used sweetness as a primary characteristic to differentiate among cultivars and preferred sweeter cultivars (Mkanda et al., 2007; Wszelaki et al., 2005). The human palate perceives different sugars to be more or less sweet. One mol of glucose is as sweet as 0.74 mol of sucrose, whereas 1 mol of fructose is as sweet as 1.17 mol of sucrose (Joesten et al., 2007). We calculated a sweetness index that incorporates the mass and relative sweetness of each sugar in whole fruit. In all cultivars examined, the sweetness index decreased rapidly with time after flowering (Fig. 4). This decline in sweetness was primarily caused by decreases in fructose and glucose in pods (Figs. 2 and 3). The mean sweetness index for 'Hystyle' was greater than that for 'Puebla 152', with the sweetness index of the other three snap beans being intermediate (Fig. 4). The linear regression coefficients for decrease in sweetness index with increasing days after flowering were significant for all cultivars, but the slopes were not significantly different from each other. These data highlight why fresh market consumers prefer smaller sieve snap beans as they perceive them to be sweeter and thus have a higher culinary value. A challenge for growers is to balance increases in yield that occur as fruit become larger against decreased sweetness and consumer acceptance. This challenge can be mitigated to some degree for snap beans by breeding for greater fruit set and harvesting fruit at a smaller sieve size. The rate of change in sweetness as pods mature is the same across cultivars. From a practical plant breeding perspective, these results suggest that selection for sweetness among genotypes could be effective at any uniform time of pod development, as long as that time was consistent for all genotypes.

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