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Changes in Carbohydrate Content and the Activities of Acid Invertase, Sucrose Synthase and Sucrose Phosphate Synthase in Vegetable Soybean During Fruit Development

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Abstract: This study investigated the changes in carbohydrate content and activities of acid invertase, sucrose synthase (SS) and sucrose phosphate synthase (SPS) in two vegetable soybean cultivars (*Glycine max* (L.) Merr. vars. Ajigen and Fuuki) during fruit development ranging from 28 to 63 days after anthesis. In both cultivars, sucrose was the predominant sugar while fructose and glucose were found in trace amounts. Sucrose accumulation was highest at 35 and 42 days after anthesis in Fuuki and Ajigen, respectively. On the other hand, fructose and glucose were almost maintained throughout the experimental period. The activity of soluble acid invertase was highest at the 42 days but was not maintained until the 63 days after anthesis. The acid invertase activity in cell wall-bound fraction was highest in young fruit (28 days after anthesis) and gradually decreased throughout development. Ajigen had higher activity than Fuuki. SS activity showed a continuous increase with time while SPS activity did not show specific inclining or declining pattern. SS and SPS activities in Fuuki were higher than Ajigen. There was a highly significant negative correlation observed between the acid invertase activity in cell wall-bound fraction and sucrose content in Ajigen. A significant positive correlation was also found between the SS and SPS activities and sucrose content in Fuuki. However, a highly significant negative correlation was observed between SS activity and other soluble sugars (glucose and fructose) in both cultivars except glucose content in Fuuki. No significant correlation was found between the SPS activity and other soluble sugars (glucose and fructose) in both cultivars.

Key words: Acid invertase, fruit development, sucrose phosphate synthase, sucrose synthase, sugar content, vegetable soybean

INTRODUCTION

Vegetable soybean (*Glycine max* (L.) Merr.) is harvested when the seeds are at immature R6 stage. The botany of vegetable soybean is similar to the field soybean except for minor morphological and physiological differences[1]. Vegetable soybean is popular in Japan, Korea, China and Taiwan, and consumption is increasing rapidly. It has excellent potential for enriching the human diet, rich source of vitamin A and a good source of carbohydrate, protein and iron[2]. Vegetable soybean is consumed mainly as a snack, but it is also used as a vegetable, an addition to soups, or processed into sweets[3]. Flavor, sweetness, texture, nutritional value and visual appearance are key quality components of vegetable soybean. Sucrose, glutamic acid and alanine are important for flavor, while sweetness is influence by the sugar levels in the seeds. Furthermore, harvest timing commonly affects seed texture.

Sugars are one of the biochemical components of fruit quality. The kind and amount of sugars directly influence fruit components. Therefore, it is important to elucidate the enzymes of sugar metabolism. Growth of soybean reproductive structures requires translocation of photosynthate from leaves to the developing fruit[4]. In plant tissue, sucrose is synthesized by sucrose phosphate synthase (SPS; EC 2.4.1.14) and degraded by invertase (EC 3.2.1.26) and sucrose synthase (SS; EC 2.4.1.13)[5]. Copeland[6] reported that cleavage by invertase is irreversible and generates glucose and fructose, whereas SS requires uridine diphosphate (UDP) as a co-substrate and produces UDP-glucose and fructose. Sucrose phosphate synthase from pea seeds was not significantly inhibited by sucrose. The range of concentrations used was wide enough to include those likely to occur *in vivo*[7]. It has been shown that SPS activity exhibits diurnal fluctuation in soybean cotyledons. SPS activity appears to be one of the limiting factors for sucrose formation in mature, photosynthetic leaves of soybean[8,9].

Many studies have been reported on enzyme activities and soluble sugars in soybean leaves,

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cotyledon, seed coat and embryo during development. However, less work has been done in intact vegetable soybean seed. Therefore, this study was conducted to investigate the enzymatic basis of soluble sugar accumulation in vegetable soybean during fruit development. The information obtained in this study could lead to novel selection criteria or molecular genetic strategies to improve vegetable soybean soluble sugar levels.

MATERIALS AND METHODS

Plant material: Two vegetable soybean cultivars (Ajigen and Fuuki) were grown in the field at the Faculty of Agriculture, Kagawa University from May to August, 2003. The pods were randomly harvested from the plot at 28, 35, 42, 49, 56 and 63 days after anthesis. Each pod of both cultivars was shelled and 10 seeds were collected for each replication. The seeds were weighed and stored at -30°C until analysis.

Enzyme extraction: Extraction of enzymes are established using the methods similar to Islam *et al.*[10]. Approximately 5 g of fresh-weight seed sample were added with 1% of polyvinylpolypyrrolidone (PVPP) and 1g sea sand. The mixture was homogenized using a cooled mortar and pestle with 5 mL of 0.2 M citrate-phosphate (C-P) buffer at pH 5.0 for acid invertase while 10 mL of 0.3 M K-P buffer (pH 7.8) containing 1 M ascorbate, 1 M $MgCl_2$, 1 M DTT and 0.1 M EDTA was used for the extraction of SS and SPS. The homogenate was then filtered through four layers of cotton cloth and the filtrate was centrifuged at 12, 000 x g, at 2°C for 10 and 20 min for acid invertase and SS and SPS, respectively. For acid invertase determination, the total supernatant was dialyzed with 0.2 M C-P buffer (pH 5.0), diluted 40 times for 12 h and the inner solution was designated as soluble fraction (SF). The residual tissues were re-extracted with 5 mL of 0.2 M NaCl C-P buffer (pH 5.0) for about 24 h with constant stirring. The supernatant was dialyzed as described above. The dialyzed solution was designated as cell wall-bound Fraction (CWBF). On the other hand, the total supernatant was dialyzed with 0.3 M K-P buffer (pH 7.8), diluted 40 times for 12 h and the inner solution was used as the crude enzyme for SS and SPS. These extractions were carried out under 0-4°C.

Enzyme assay: The standard assay medium for acid invertase consisted of 0.2 mL of 0.2 M C-P buffer (pH 5.0), 0.1 mL of 0.5 M sucrose, 0.1 mL of distilled water and 0.1 mL of crude enzyme solution. The blank experiment contained distilled water instead of sucrose. The assay mixture was incubated at 45°C for 15 min. After the reaction, the assay mixture was neutralized with 0.1 N NaOH or 0.1 N HCl and added with a coloring Somogyi's copper reagent. The mixture was heated for 10 min in boiling water. The amount of reducing sugars was estimated by the method of Somogyi[11]. Soluble protein content was determined by Lowry[12] method using bovine serum albumin as the standard. The enzyme activity was expressed as the amount of glucose produced per minute per milligram of protein.

SS and SPS activities were assayed at 37°C by the method described by Hubbard[13] with slight modifications. Reaction mixtures (70.75 μL) contained 50 mM Hepes-NaOH buffer (pH 7.5), 15 mM $MgCl_2$, 25 mM fructose-6-phosphate, 25 mM glucose-6-phosphate and 25 mM UDP-glucose. The mixtures were incubated for 30 min at 37°C and the reaction was terminated with the addition of 70 μL of 30% KOH. Tubes were kept at 100°C for 10 min to destroy any unreacted fructose or fructose-6-phosphate. After cooling, 2 mL of anthrone reagent (150 mg anthrone in 100 mL of 70% H_2SO_4) was added and incubated in a 40°C water bath for 15 min. After cooling, color development was measured at OD 620 nm. SS was assayed as above but with 25 mM fructose instead of fructose-6-phosphate and in the absence of glucose-6-phosphate. The soluble protein content was determined by the method of Lowry[12] using bovine serum albumin as the standard. The enzyme activity was measured as micromole of sucrose or sucrose-6-phosphate produced per minute per milligram of protein.

Carbohydrate determination: Soluble sugars, sucrose, glucose and fructose were determined by HPLC, as previously reported by Islam *et al.*[10]. Approximately 2 g of fresh-weight seeds sample was mixed with 1 g of sea sand and homogenized in a cooled mortar and pestle. Ten milliliter of distilled water was added to the homogenate and centrifuged at 12,000 x g, at 2°C for 10 min. The mixture was filtered through a cellulose nitrate membrane filter (0.2 μm pore size). Soluble sugars were analyzed by HPLC using a stainless steel column (10.7 mm IDx30.0 cm) packed with silica gel (gel pack C610). The mobile phase (filtered water) was pumped through the column at a flow rate of 1.0 mL min^{-1}. The pressure was adjusted to 14-15 kg cm^{-2} and the temperature to 60°C. A RI monitor (Hitachi L-3300) was used. Sucrose, glucose and fructose were identified by their retention times and were quantified according to standards.

Data analysis: A randomized complete block design with three replications was adopted. The level of significance was calculated from the *F*-value of ANOVA. The relationship between sugars and enzyme activities were described with linear correlation analysis.

RESULTS

Seed development: Figure 1 showed that the weight increase gradually throughout the experimental period. Comparing the two cultivars, Fuuki showed a higher fresh weight than Ajigen.

Acid invertase activity: The acid invertase activity in soluble fraction was highest at the 42 days but was not maintained until the 63 days after anthesis. Fuuki has a commutatively lower acid invertase activity than Ajigen (Fig. 2). On the other hand, invertase activity in cell wall-bound fraction in both cultivars gradually decreased with few fluctuations as the fruit matures. Higher enzyme activity was found in Ajigen than Fuuki.

Sucrose synthase and sucrose phosphate synthase activities: A continuous increase with time in SS activity was observed in both cultivars (Fig. 3). Fuuki showed higher activity than Ajigen except after the 56 days after anthesis.

There was no specific inclining or declining pattern of SPS activity in both cultivars (Fig. 3). In case of Fuuki, it showed a wavy pattern with a sharp increase on 35 days

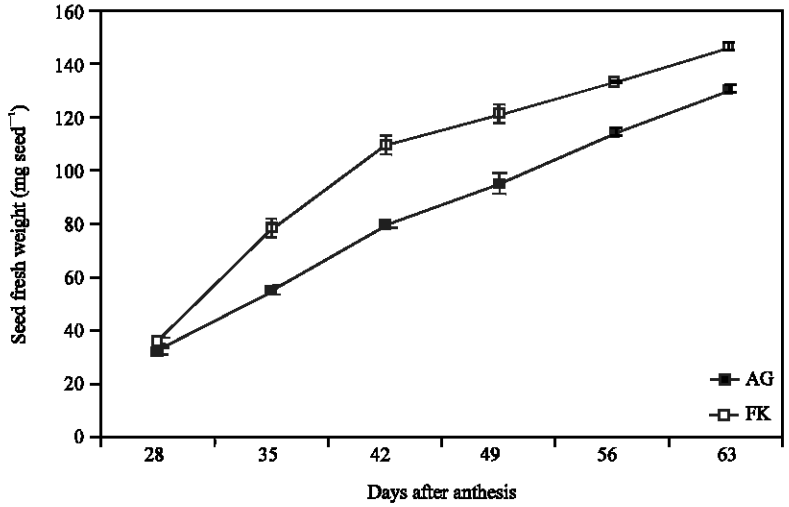

Fig. 1: Seed fresh weight of two vegetable soybean cultivars (Ajigen and Fuuki) during seed development. Each point represents the mean of 3 replications. Vertical bars indicate SE. SE bars were not shown when masked by the graph symbols. AG = Ajigen; FK = Fuuki

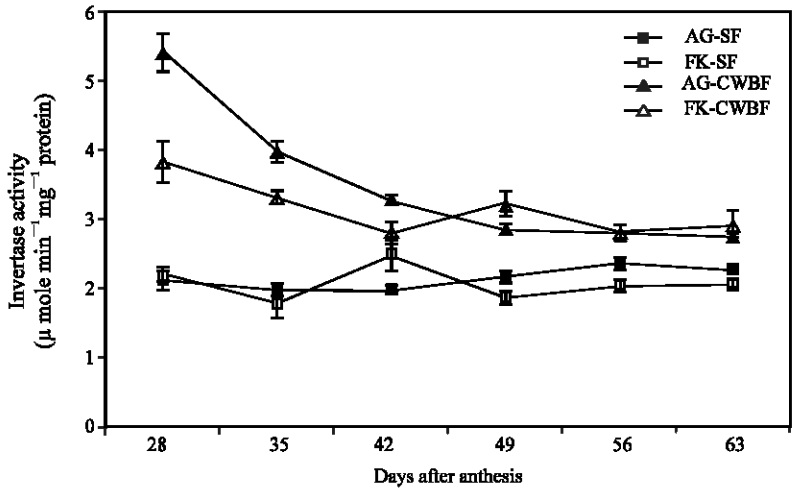

Fig. 2: Changes in acid invertase activity in the soluble faction (SF) and cell wall-bound faction (CWBF) of two vegetable soybean cultivars (Ajigen and Fuuki) during seed development. Each point represents the mean of 3 replications. Vertical bars indicate SE. SE bars were not shown when masked by the graph symbols. Legend as shown in Fig. 1

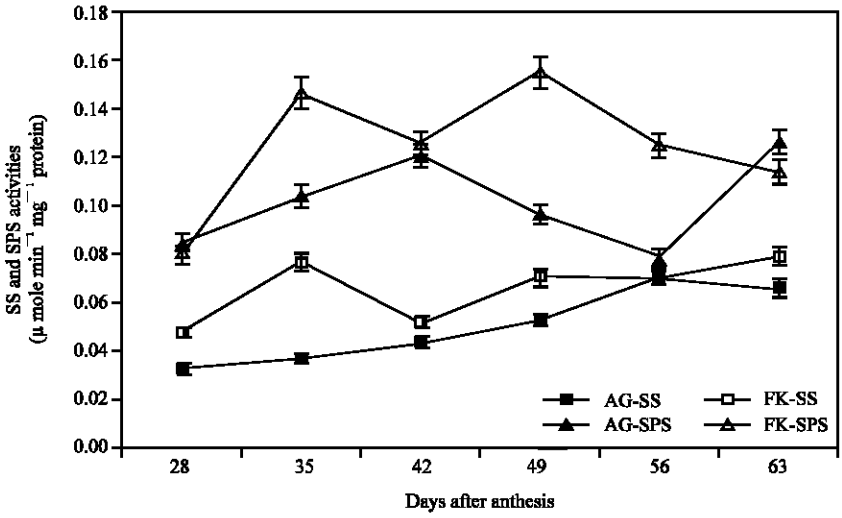

Fig. 3: Changes in sucrose synthase (SS) and sucrose phosphate synthase (SPS) activities of two vegetable soybean cultivars (Ajigen and Fuuki) during seed development. Each point represents the mean of 3 replications. Vertical bars indicate SE. SE bars were not shown when masked by the graph symbols. Legend as shown in Fig. 1

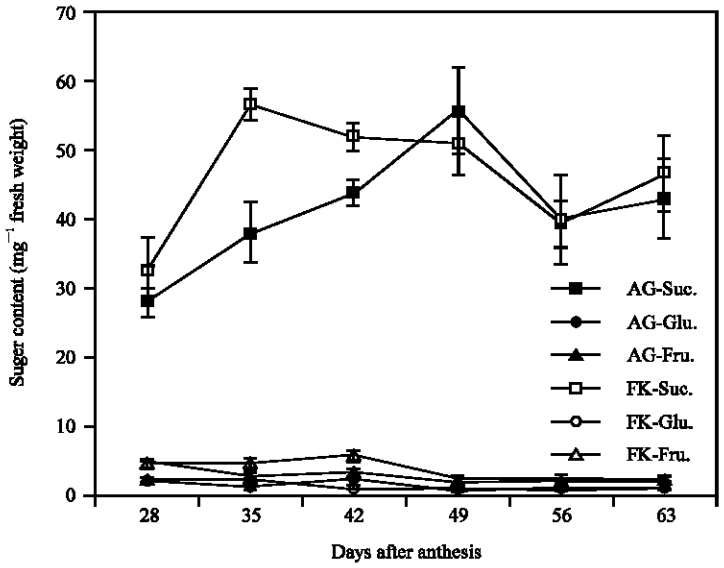

Fig. 4: Changes in soluble sugar contents of two vegetable soybean cultivars (Ajigen and Fuuki) during seed development. Each point represents the mean of 3 replications. Vertical bars indicate SE. SE bars were not shown when masked by the graph symbols. Legend as shown in Fig. 1

while in Ajigen, the activity reached a maximum on the 42 days and fluctuated thereafter.

Changes in soluble sugar content: The highest sucrose content was observed on the 35 and 49 days after anthesis in Fuuki and Ajigen, respectively (Fig. 4). A higher sucrose content was observed in Fuuki than Ajigen. Fructose and glucose contents, on the other hand, did not significantly change in both cultivars throughout the fruit development stage.

Correlation coefficients *(r)* between enzyme activities and sugar contents: There was a highly significant negative correlation observed between the acid invertase activity in cell wall-bound fraction of Ajigen and sucrose content. On the other hand, a significant positive correlation was

Table 1: Correlation coefficient *(r)* values between enzyme activities and sugar contents of two vegetable soybean cultivars during seed development

		Correlation coefficient value			
		Acid invertase			
Sugar content	Cultivar	SF[a]	CWBF[b]	SS[c]	SPS[d]
Sucrose	Ajigen	0.086	-0.790**	0.366	0.304
	Fuuki	-0.298	-0.381	0.528*	0.830**
Glucose	Ajigen	-0.534*	0.509*	-0.507*	0.193
	Fuuki	-0.217	0.858**	-0.161	-0.306
Fructose	Ajigen	-0.389	0.901**	-0.672**	-0.172
	Fuuki	0.519*	0.243	-0.536*	-0.231

[a]SF: Soluble fraction, [b]CWBF: Cell wall-bound fraction,
[c]SS: Sucrose synthase, [d]SPS: Sucrose phosphate synthase
*,**denote significant correlation at $p<0.05$ and $p<0.01$, respectively, n=18

found between the SS and SPS activities and sucrose content in Fuuki. However, a highly significant negative correlation was observed between SS activity and other soluble sugars (glucose and fructose) of each cultivar except glucose content of Fuuki. No significant correlation was found between the SPS activity and other soluble sugars (glucose and fructose) in both cultivars (Table 1).

DISCUSSION

The fruit weight gradually increase throughout the experimental period in both cultivars. This pattern is similar to that observed in tomato during fruit development[10] that fruit weight increase after anthesis due to rapid cell expansion accompanied by rapid synthesis of large amount of both starch and storage proteins in the cotyledons. Lowell and Kuo[14] reported that sucrose synthase is generally greater in sink tissues and have been associated with dry matter accumulation in soybean seeds.

Acid invertase activity in the soluble fraction increased during fruit development. The activity was highest at the 42 days but was not maintained until the 63 days after anthesis. Fuuki has a commutatively lower acid invertase activity than Ajigen. During the early stage of fruit development, acid invertase was apparently low enough to allow sucrose to accumulate. In case of muskmelon fruit, McCollum *et al.*[15] reported that the increasing sucrose concentration could be due to increased sucrose synthase activity accompanied by a decrease in acid invertase activity during fruit growth. Sucrose degrading enzymes, also play a developmental role in sucrose accumulation. On the other hand, invertase activity in cell wall-bound fraction in both cultivars gradually decreased as the fruit matures. Similar pattern was found in fava bean cotyledon during development[16]. Kivilaan *et al.*[17] associated invertase activity with cell wall formation of carrot protoplasts by the action of cellulase and pectines accompanied by the release of 50 to 60% of the invertase activity from the cells[18]. Shibles *et al.*[4] reported that during early fruit development in soybean, the pod wall develops more rapidly than the seed. Consequently, utilization of translocated photosynthate by the developing pod walls may be necessary for pod growth before the seed begins to develop. Many studies reported that invertase activity is greatest during the early stages of embryo and fruit development in maize liquid endosperm, fruits of watermelon, pepper and tomato[19-22].

SS activity showed a continuous increase with time during fruit development in both cultivars. Gross and Pharr[23] reported that a metabolic pathway for hydrolysis of stachyose and raffinose with subsequent conversion of galactose to sucrose in the fruit pedicel of several stachyose translocating species. SS was implicated in the synthesis of sucrose because high activity was observed in the pedicel of cucumber[23]. Accordingly, it was postulated that sucrose was the predominant sugar of translocation in vegetable soybean. On the other hand, there was no specific inclining or declining pattern of SPS activity in both cultivars. In case of Fuuki, it showed a wavy pattern with a sharp increase on 35 days while in Ajigen, the activity reached a maximum on the 42 days and fluctuated thereafter. Kuo *et al.*[24] suggested that the presence of SPS activity in the seed coat that monosaccharides derived from invertase activity can be converted to sucrose (in combination with sucrose phosphate phosphatase) for cellular metabolism and/or transport to the embryo. This pattern is similar to that of maturing soybean seed tissues[24].

Vegetable soybean contain higher level of sucrose than glucose and fructose during fruit development. The highest sucrose content was observed at 35 and 49 days after anthesis in Fuuki and Ajigen, respectively. Fructose and glucose were found in trace amounts and did not significantly change in both cultivars throughout the fruit development stage. Previous study also found similar pattern in maturing soybean seed tissues[24] and soybean cotyledon during germination[25]. Ackerson[26] suggested that due to the major translocated sugar is sucrose, the utilization of sucrose by developing embryo may be a yield-determining process in soybean. Moreover, sucrose is the primary carbon source translocated from leaf tissues to the growing soybean embryos[27]. It is a precursor of raffinose saccharides, known to be the sugar imported into developing soybean seeds[28]. During seed filling, photo assimilates are translocated as sucrose via the phloem to the seed[16]. Rates of sucrose uptake in the embryo decline with physiological maturity[29]. Soybean

seeds also have the capacity to convert ^{14}C-glucose to sucrose[30]. Sung *et al.*[31] reported initial sucrose catabolism in the embryo is to provide carbon for seed storage product and respiration. It could be catalyzed by SS, although the contribution of SS can vary with respect to tissue type, function and age. Kawamura and Tada[32] suggested the majority of soluble carbohydrates are oligosaccharides, including sucrose and the raffinose series (raffinose, stachyose and trace amount of verbascose) that are found in cotyledon, seed coat and hypocotyl. The concentrations of glucose and fructose in seed tissues may suggest that invertase is functional in the seed coat[24]. Photosynthates were transferred from the seed coat to embryo primarily as sucrose with only 1% converted to reducing sugars by the seed coat[33,34]. Early in seed development, concentrations of glucose and fructose are high but decline as seed matures[35].

There was a highly significant negative correlation between the acid invertase activity of cell wall-bound fraction of Ajigen and sucrose content. A highly positive correlation between SS activity and sucrose content was also observed in Fuuki. This result suggests that SS was in the direction of sucrose synthesis period. In most instances, it is assumed that SS is responsible for sucrose degradation. Although, in some plant tissues, it has been reported that SS could act in the direction of sucrose synthesis[10,36] which supported this finding. It catalyzes a reversible reaction and under normal condition *in vivo*, it acts only in the breaking down of sucrose[5].

Based on the results obtained, it is concluded that there are physiological and compositional changes occurred during fruit and seed development toward maturity. It appears that seed fresh weight, soluble acid invertase and SS activity gradually increase while the activity of acid invertase gradually decreased in cell wall-bound fraction throughout the experimental period. The highest sucrose content was observed at 35 and 49 days after anthesis in Fuuki and Ajigen, respectively. Sucrose synthase was implicated in the synthesis of sucrose. Fuuki showed higher sucrose content and enzyme activities (SS and SPS) than Ajigen except in acid invertase (SF and CWBF).

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REFERENCES

1. Konovsky, J., T.A. Lumpkin, S. Shanmugasundaram and T.S.C. Tsou, 1992. Edamame: The Vegetable Soybean. Asian Vegetable Research and Development Center, Tainan, Taiwan.
2. Lal, G., S.H. Lai and S. Shanmugasundaram, Unpublished. AVRDC Training Guide: Suggested Cultural Practices for Vegetable Soybean. Asian Vegetable Research and Development Center, Tainan, Taiwan. http://www.avrdc.org
3. Konovsky, J., T.A. Lumpkin and D. McClary, 1994. Edamame: The Vegetable Soybean. In: Understanding the Japanese Food and Agrimarket: A Multifaceted Opportunity (Ed. O´Rourke, A.D.), Haworth Press, Binghamton, pp: 173-181.
4. Shibles, R.M., I.C. Anderson and A.H. Gibson, 1975. Some Case Histories. In: Crop Physiology (Ed. Evans, L.T.), Cambridge University Press, pp: 151-189.
5. Hurst, P.L., L.M. Hyndman and P.J. Hannan, 1993. Sucrose synthase, invertase and sugars in growing asparagus spears. NZ J. Crop Hortic. Sci., 21: 331-336.
6. Copeland, L., 1990. Enzymes of Sucrose Metabolism. In: Methods in Plant Biochem., Enzymes of Primary Metabolism (Ed. Lea, P.J.), Academ. Press, London, pp: 73-86.
7. Gerhardt, R., M. Stitt and H.W. Heldt, 1987. Subcellular metabolite levels in spinach leaves. Plant Physiol., 83: 399-407.
8. Huber, S.C. and D.W. Israel, 1982. Biochemical basis for partitioning of photosynthetically fixed carbon between starch and sucrose in soybean (*Glycine max* Merr.) leaves. Plant Physiol., 69: 691-696.
9. Huber, S.C., 1983. Role of sucrose-phosphate synthase in partitioning of carbon in leaves. Plant Physiol., 71: 818-821.
10. Islam, M.S., T. Matsui and Y. Yoshida, 1996. Carbohydrate content and the activities of sucrose synthase, sucrose phosphate synthase and acid invertase in different tomato cultivars during fruit development. Sci. Hortic., 65: 125-136.
11. Somogyi, M., 1952. Notes on sugar determination. J. Biol. Chem., 195: 19-23.
12. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
13. Hubbard, N.L., S.C. Huber and D.M. Pharr, 1989. Sucrose phosphate synthase and acid invertase as determinants of sucrose concentration in developing muskmelon (*Cucumis melo* L.) fruits. Plant Physiol., 91: 1527-1534.

14. Lowell, C.A. and T.M. Kuo, 1989. Oligosaccharide metabolism and accumulation in developing soybean seeds. Crop Sci., 29: 459-465.
15. McCollum, T.G., D.J. Huber and D.J. Cantliffe, 1988. Soluble sugar accumulation and activity of related enzymes during muskmelon fruit development. J. Am. Soc. Hortic. Sci., 113: 399-403.
16. Weber, H., L. Borisjuk, U. Heim, P. Buchner and U. Wobus, 1995. Seed coat-associated invertases of fava bean control both unloading and storage function: cloning of cDNAs and cell type-specific expression. Plant Cell, 7: 1835-1846.
17. Kivilaan, A., T.C. Beaman and R.S. Bandurski, 1961. Enzymatic activities associated with cell wall preparations from corn coleoptiles. Plant Physiol., 36: 605-610.
18. Ueda, Y., H. Ishiyama, M. Fukui and A. Nishi, 1974. Invertase in cultured *Daucus carota* cells. Phytochemistry, 13: 383-387.
19. Tsai, C.Y., F. Salamini and O.E. Nelson, 1970. Enzymes of carbohydrate metabolism in the developing endosperm of maize. Plant Physiol., 46: 299-306.
20. Manning, K. and G.A. Maw, 1975. Distribution of acid invertase in the tomato plant. Phytochemistry, 14: 1965-1969.
21. Walker, R.R. and J.S. Hawker, 1976. Effect of pollination on carbohydrate metabolism in young fruits of *Citrullus lanatus* and *Capsicum annuum*. Phytochemistry, 15: 1881-1884.
22. Russell, C.R. and D.A. Morris, 1982. Invertase activity, soluble carbohydrates and inflorescence development in the tomato (*Lycopersicon esculentum* Mill.). Ann. Bot., 49: 89-98.
23. Gross, K.C. and D.M. Pharr, 1982. A potential pathway for galactose metabolism in *Cucumis sativus* L., a stachyose transporting species. Plant Physiol., 69: 117-121.
24. Kuo, T.M., C.A. Lowell and P.T. Smith, 1997. Changes in soluble carbohydrates and enzymic activities in maturing soybean seed tissues. Plant Sci., 125: 1-11.
25. Kuo, T.M., D.C. Doehlert and C.G. Crawford, 1990. Sugar metabolism in germinating soybean seeds. Plant Physiol., 93: 1514-1520.
26. Ackerson, R.C., 1985. Invertase activity and abscisic acid in relation to carbohydrate status in developing soybean reproductive structures. Crop Sci., 25: 615-618.
27. Thorne, J.H., 1980. Kinetics of ^{14}C-photosynthate uptake by developing soybean fruit. Plant Physiol., 65: 975-979.
28. Thorne, J.H., 1985. Phloem unloading of C and N assimilates in developing seeds. Ann. Rev. Plant Physiol., 36: 317-343.
29. VerNooy, C.D., J.H. Thorne, W. Lin and R.M. Rainbird, 1986. Cessation of assimilate uptake in maturing soybean seeds. Plant Physiol., 82: 222-225.
30. Hageman, M.E. and R.W. Rinne, 1976. Glucose, pyruvate and acetate metabolism by developing soybean seeds. Plant Cell Physiol., 17: 501-507.
31. Sung, S.S., D. Xu and C.C. Black, 1989. Identification of actively filling sucrose sinks. Plant Physiol., 89: 1117-1121.
32. Kawamura, S. and M. Tada, 1967. Isolation and determination of sugars from the cotyledon, hull and hypocotyl of soybeans by carbon column chromatography. Tech. Bull. Fac. Agric. Kagawa Univ., 18: 138-141.
33. Thorne, J.H. and R.M. Rainbird, 1983. An *in vivo* technique for the study of phloem unloading in seed coats of developing soybean seeds. Plant Physiol., 72: 268-271.
34. Doehlert, D.C. and F.C. Felker, 1987. Characterization and distribution of invertase activity in developing maize (*Zea mays*) kernels. Physiol. Plant., 70: 51-57.
35. Yazdi-Samadi, B., R.W. Rinne and R.D. Seif, 1977. Components of developing soybean seeds: Oil, protein, sugars, starch, organic acids and amino acids. Agron. J., 69: 481-486.
36. Moriguchi, T., T. Sanada and S. Yamaki, 1990. Seasonal fluctuations of some enzymes relating to sucrose and sorbitol metabolism in peach fruit. J. Am. Soc. Hortic. Sci., 115: 278-281.