Sugar Metabolism in Germinating Soybean Seeds

Evidence for the Sorbitol Pathway in Soybean Axes

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ABSTRACT

Characterization of sugar content and enzyme activity in germinating soybean (Glycine max L. Merrell) seeds led to the discovery of sorbitol accumulating in the axes during germination. The identity of sorbitol was confirmed by relative retention times on high-performance liquid chromatography and gas liquid chromatography and by mass spectra identical with authentic sorbitol. Accumulation of sorbitol in the axes started on day 1 of germination as sucrose decreased and glucose and fructose increased. Sucrose also decreased in the cotyledons, but there was no accumulation of sorbitol, glucose, or fructose. Accumulation of sorbitol and hexoses was highly correlated with increased invertase activity in the axes, but not with sucrose synthase and sucrose phosphate synthase activities. Sucrose synthase activity was relatively high in the axes, whereas the activity of sucrose phosphate synthase was relatively high in the cotyledons. Ketose reductase and aldose reductase were detected in germinating soybean axes, but not in cotyledons. Fructokinase and glucokinase were present in both axes and cotyledons. The data suggest a sorbitol pathway functioning in germinating soybean axes, which allows for the interconversion of glucose and fructose with sorbitol as an intermediate.

Sorbitol (D-glucitol) is an acyclic polyol found in a number of plant species, but most abundant in the Rosaceae family (22). A primary photosynthetic product in apples, pears, and stone fruits that accounts for 60 to 90% of the carbon transported from the leaf, sorbitol is present in amounts ranging up to 10% on a fresh weight basis in many of these rosaceous fruits (2, 15). It is also found in petiole, bark, and wood of both stem and root tissues of apple seedlings (6) and intact seeds (23). The distribution of sorbitol in apple seedlings is affected by seasonal and daily changes (6). In corn, sorbitol is found in seed and silk but not in pollen or leaf (5). Low amounts of sorbitol are detectable in developing corn kernels that apparently are not translocated from leaf tissues (20).

The metabolism of sorbitol has been extensively studied in leaves, fruits and callus tissues of several woody Rosaceae species (2, 15). The major enzymic activities involved in sorbitol metabolism in apples include aldose 6-phosphate reductase and sorbitol phosphatase for the synthesis of sorbitol, and NAD^+-dependent sorbitol dehydrogenase for the conversion of sorbitol to fructose (17, 18). Apple leaves also contain $NADP^+$-dependent sorbitol dehydrogenase for the interconversion between sorbitol and fructose (24). Changes in these enzyme activities have been described in developing apple leaves (16) and fruits (1), and in Japanese pear fruits during development and maturation (25). Recently, a ketose reductase, which catalyzes the NADH-dependent interconversion between fructose and sorbitol, has been isolated and partially characterized from developing maize endosperm (9). No information concerning the occurrence and metabolism of sorbitol in soybean tissues has been available.

The objectives of this study were to characterize sugar metabolism in germinating soybean seeds. The detection and positive identification of sorbitol in germinating soybean axes prompted further studies to investigate the role of sorbitol in sugar metabolism. In the present paper, we report finding sorbitol in germinating soybean axes in association with invertase activity. Both ketose reductase and aldose reductase, two enzymes involved in interconversion of glucose and fructose with sorbitol as an intermediate, have been detected in crude extracts of axes, but not cotyledons where no sorbitol accumulation was found. Additional information on the mechanisms of sucrose and hexose metabolism in germinating soybean seeds is also provided.

MATERIALS AND METHODS

Seed Source and Chemicals

Soybean (Glycine max L. Merrill cv Williams 82) seeds were purchased from Kelly Seed Co.[1], Peoria, IL. Preswollen microgranular DE 52 and CM 52 were obtained from Whatman (Clifton, NJ). Uniflo membrane (0.2 μm) was from Schleicher & Schuell (Keene, NH). Ultrapure grade of authentic sugar standards used in HPLC and GC-MS analyses were acquired from Pfanstiehl Laboratories (Waukegan, IL), except that L-iditol was from Aldrich (Milwaukee, WI). Dithiothreitol, Hepes, and Mops were purchased from Research Organics (Cleveland, OH). Other chemicals were obtained from Sigma (St. Louis, MO). Unless indicated, water used in this study was distilled and further purified by a Nanopure II ion-exchange system (Sybron/Barnstead; Boston, MA).

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[1] The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other similar products not mentioned.

Germination and Sample Preparation

About 50 g of soybean seeds were washed in 0.5% sodium hypochlorite for 15 min, rinsed thoroughly with deionized water and imbibed in water for 6 h at room temperature (20–22°C). About 600 imbibed seeds were separated into cotyledons and embryonic axes for use as the zero-time control sample. Fifty intact cotyledon pairs and all the remaining embryonic axes were lyophilized for sugar analysis. Their fresh and dry weights were recorded. Between 50 and 75 seeds of uniform size were placed on wetted filter paper in a plastic Petri dish (100 × 15 mm) with eight openings (65 mm diameter) evenly distributed on the cover for aeration. Seeds were germinated in the dark in an incubation chamber maintained at 25 to 27°C. Each day, seeds from two to four dishes were rinsed with water, then separated into cotyledons and embryonic axes. Tissues were then treated as described below for enzyme and sugar analyses.

Sugar Analysis

Lyophilized embryonic axes and cotyledons were ground into fine powder, defatted with hexane for 5 to 6 h in a Soxhlet extractor, and air-dried. Subsequent sugar extraction, HPLC analysis, and quantitation were carried out by the procedures described previously (14) with some modifications. The sugar extracts were obtained from 150 to 250 mg defatted meal in 10 mL 80% aqueous ethanol at 75°C for 30 min. The extracts, after centrifugation, were passed through a column of 1 mL CM 52 overlaid by 1.5 mL DE 52, to remove ionic substances. For sugar analysis, an additional guard column (Waters Guard Column Kit; Milford, MA) packed with Ca^{2+}-loaded resin (Interaction Chemical; Mountain View, CA) was installed between the analytical column (Sugar Pak I; Waters Associates, Milford, MA) and a cation guard cartridge (Pierce; Rockford, IL). Both main and guard columns were maintained at 90°C, whereas the cation cartridge was at room temperature.

Enzyme Extraction and Assays

Extraction of enzymes was conducted at 4°C. Enzyme extracts were obtained from 0.3 g chopped, fresh tissues in 3 mL 50 mM Mops-HCl (pH 7.2), containing 5 mM $MgCl_2$, 3 mM DTT, 0.5% Triton X-100 (v/v), and 0.5 M NaCl, homogenized with a Brinkman Polytron tissue homogenizer (Westbury, NY) for 1 min. A portion of the homogenate was used to assay for both soluble and insoluble invertase. The remaining homogenate was centrifuged at 27,000g for 15 min, and the clear supernatant was used to assay for the activities of sucrose synthase and sucrose phosphate synthase. Due to the unstable nature of sucrose phosphate synthase, the clear supernatant obtained after centrifugation was used directly for the measurement of activities. For the assay of glucokinase, fructokinase, aldose and ketose reductases, enzymes were extracted from lyophilized soybean cotyledons and axes harvested after 2 d of germination. The tissue (0.25 g) was homogenized with a Polytron in 6 mL of extraction buffer containing 50 mM Hepes-NaOH (pH 7.5), 5 mM $MgCl_2$, and 1 mM DTT. After centrifugation at 20,000g for 20 min and

prior to enzyme assay, the clear supernatant was dialyzed overnight at 4°C against two changes of buffer (2 L) containing 10 mM Hepes-NaOH (pH 7.5), 5 mM $MgCl_2$, and 1 mM DTT.

Combined soluble and insoluble invertase (EC 3.2.1.26) activity was assayed at 30°C for 15 min in a reaction mixture containing enzyme extract and 100 mM sucrose in 200 mM sodium acetate (pH 5.0). The amount of glucose produced in the assay was determined by a glucose oxidase assay method, as described by Gascon and Lampen (12).

Sucrose synthase (EC 2.4.1.13) activity was assayed for sucrose produced at 30°C for 15 to 20 min in the presence of 12 mM fructose and 12 mM UDP-Glc in 50 mM Hepes-NaOH (pH 7.5), according to the procedure of Cardini et al. (4). This procedure was also used to assay the activity of sucrose phosphate synthase (EC 2.4.1.14), but the reaction mixture contained 25 mM fructose 6-phosphate, 10 mM UDP-Glc, 10 mM $MgCl_2$, and 10 mM phenyl β-glucoside in 150 mM Hepes-NaOH (pH 7.5).

Glucokinase (EC 2.7.1.2) and fructokinase (EC 2.7.1.4) activities were measured by coupling hexose phosphate production with NAD reduction as described previously (11).

Ketose reductase (EC 1.1.1.14) activity was measured spectrophotometrically by the decrease in A_{340} in the presence of fructose (17). The reaction mixture contained 50 mM Mes-NaOH (pH 6.0), 5 mM $CaCl_2$, 0.8 mM NADH or NADPH, and 400 mM fructose. Blanks contained no fructose. Assays were initiated with the addition of hexose. Aldose reductase (EC 1.1.1.21) activity was measured in the same manner except that $MgCl_2$ and glucose replaced $CaCl_2$ and fructose, respectively, in the reaction. All enzyme assays were optimized experimentally with crude soybean extracts.

Isolation and Identification of Soybean Axis Component

The unknown compound from soybean axis was isolated by HPLC and the structure was determined by GC-MS analysis. Forty-five mL of hot 80% aqueous ethanol were added to 1.5 g of defatted fine powder of 2-d germinated soybean embryonic axes and extracted with shaking at 72°C for 30 min then reextracted twice with 15 mL 80% ethanol under the same conditions. The combined extracts were filtered through No.54 Whatman filter paper and reduced to about 10 mL on a rotary evaporator. The extract was deionized on a 2.5 × 4.5 cm column of 5 mL CM 52 overlaid by 15 mL DE 52, equilibrated with water. The first 10 mL of eluent were discarded, and the next 40 mL were collected, passed through an Uniflo filter, and lyophilized. The resulting sample was resuspended in water and passed through 15 mL DE 52. The eluent was filtered, lyophilized, and stored at −15°C.

The dry sample was dissolved in 1.2 mL water and 75 μL was analyzed by HPLC as described above, except the mobile phase was water instead of 0.1 mM Ca^{2+}·EDTA, and the temperature of both main and guard columns was maintained at 85°C. The elution was monitored by a Waters 410 differential refractometer and fractions were collected from repeated injections until about 2 mL, which was equivalent to 1 mg pure sugar, was collected. The sample was lyophilized and used without further purification for GC and GC-MS analyses. The soybean axis compound and authentic stand-
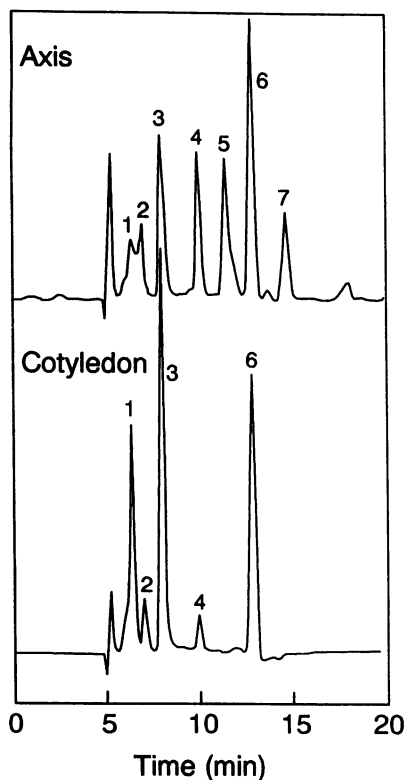
Figure 1. HPLC separation of sugars and sorbitol extracted from axes (top) and cotyledons (bottom) of 2-d germinating soybeans. The elution peaks were identified by the same retention time of each authentic standard. Mannitol was added as an internal standard prior to extraction. 1, Stachyose; 2, raffinose; 3, sucrose; 4, glucose; 5, fructose; 6, mannitol; 7, sorbitol.

ards, including polyols, glucose and fructose (0.5 mg each) were converted into acetylated derivatives by the treatment of dry samples with 0.5 mL acetic anhydride in the presence of 0.5 mL pyridine containing 45 mg/mL hydroxylamine-HCl at 60°C for 20 min, according to a procedure reported by Seymour *et al.* (19). The acetylated derivatives were then analyzed by a Perkin-Elmer Sigma 3B Capillary Chromatograph (Norwalk, CT), which was equipped with a 25 m methyl silicone capillary column (Hewlett-Packard; Palo Alto, CA) and a hydrogen-flame ionization detector. The injector and the detector temperature was set at 275°C using helium as carrier gas at 1 mL/min. The oven temperature was programmed from 160 to 185°C at 5° per min. Mass spectra were recorded by a Hewlett-Packard Mass Selective Detector (5970 Series) with a fixed electron energy voltage at 68 ± 3 eV. Other GC-MS conditions were as follows: interface temperature, 235°C; detector temperature, 200°C; split ratio, 1:100; injection volume, 0.5 μL.

Data Analyses

All data, except specified in Table V, are the mean of two separate experiments. In each experiment, two extractions were made from each sample for sugar and enzyme analyses.

Standard errors were determined for all data. Correlation coefficients were determined from the mean values.

RESULTS

The HPLC elution profiles of soluble sugars extracted from germinating soybean axes and cotyledons were distinctively different (Fig. 1). For instance, in d 2 of seed germination, axes contained much smaller amounts of stachyose and sucrose, and a much larger amount of glucose than cotyledons. Fructose and an unknown compound, labeled peak No. 7, were present in axes but absent in cotyledons (Fig. 1). The putative sorbitol was not detectable in the mature, dry seeds of 'Williams 82' (data not shown), but a substantial amount accumulated during germination. To determine the identity of the compound, material was isolated from 2-d germinating soybean axes by repetitive injections on HPLC and 11.5 mg of the unknown was obtained from 1.5 g of dry axis. A Sugar-Pak I column was used because this column was able to separate sorbitol from a mixture containing some naturally occurring six-carbon polyols, such as galactitol, L-iditol, myo-inositol and D-mannitol (Table I). In addition, the germinating soybean axes did not contain detectable D-mannitol, galactitol and L-iditol, three polyols that elute near sorbitol under these HPLC conditions. Hence, a highly purified preparation of the soybean axis compound was obtained by repetitive HPLC runs without further purification.

The soybean axis compound cochromatographed as a single sharp peak when mixed with authentic sorbitol on HPLC with a relative retention time of 1.28 (Table I). The acetylated derivative of soybean axis compound also cochromatographed with authentic sorbitol as a single peak when mixed and analyzed by GC. The six-carbon polyols discussed above were also completely separated and gave different retention times on GC (Table I). In this study, galactitol was eluted after sorbitol from a methyl silicone capillary column, although it was eluted before sorbitol from an OV-17 column, as reported by Carey *et al.* (5). Combined GC-MS of the acetylated soybean axis compound gave a mass spectrum (Fig. 2) identical to an authentic sorbitol standard. The mixture of an equal amount of the soybean axis compound and the authentic standard also gave an identical mass spectrum. Moreover, both soybean axis compound and authentic sor-

Table I. *Relative Retention Times of Some Naturally Occurring Six-Carbon Polyols on HPLC and GC*

HPLC conditions used for the isolation of soybean axis sorbitol were applied to determine the retention times. The GC procedure is described in "Materials and Methods."

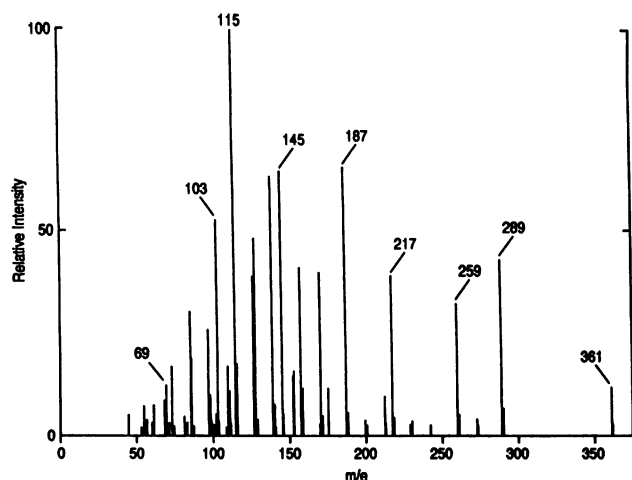| Compound	HPLC	GC
	relative retention time	
myo-Inositol	1.00	1.00
D-Mannitol	1.10	1.02
Galactitol	1.23	1.06
Sorbitol	1.28	1.04
Soybean axis compound	1.28	1.04
L-Iditol	1.41	1.07

Figure 2. Mass spectrum of acetylated soybean axis compound. Soybean axis sorbitol was acetylated with acetic anhydride to form sorbitol (D-glucitol) hexa-acetate and analyzed by a combination of Perkin-Elmer Sigma 3B Capillary Chromatograph equipped with a 25 m methyl silicone column and Hewlett-Packard Mass Selective Detector. The mass spectrum derived from the acetylated soybean axis compound is identical to that from the acetylated authentic sorbitol.

bitol were converted to fructose, as determined by HPLC, by sheep liver sorbitol dehydrogenase in the presence of NAD^+ (data not shown). The identical properties of the soybean axis compound and authentic standard on HPLC, GC, GC-MS, and enzymatic reaction therefore confirmed the identity of the soybean axis compound as sorbitol.

Soluble sugars and sorbitol were extracted from germinating cotyledons and axes, and analyzed by HPLC (Table II). Cotyledons contained sucrose, relatively low levels of glucose, and no fructose or sorbitol at d 0 of germination. Sucrose levels were fairly constant during d 0, 1, and 2, but declined in d 3 and 4. Glucose remained fairly constant, as fructose and sorbitol remained absent throughout this period. Like cotyledons, germinating soybean axes also contained sucrose,

low levels of glucose but no fructose or sorbitol at d 0. Sucrose levels were highest at d 1 and declined thereafter. Unlike cotyledons, however, germinating soybean axes rapidly accumulated glucose, fructose, and sorbitol through d 2, after which levels remained constant at d 3 and declined somewhat at d 4.

Enzymes of sucrose metabolism were assayed from the same samples used for the analysis of soluble carbohydrates. In cotyledons, invertase activity was present in low but detectable levels throughout the examined period of germination (Table III). Sucrose synthase activity was not detectable at d 0 and 1 but increased to a relatively low level thereafter, whereas sucrose phosphate synthase activity was present at a relatively constant level. Activities of these enzymes in germinating soybean axes, however, exhibited different patterns from those observed in cotyledons. In axes, invertase activity increased rapidly from d 0 to d 2 and remained at fairly high levels thereafter. Sucrose synthase activity was present at fairly high levels during the first 2 d of germination and declined at d 3 and 4, whereas the activity of sucrose phosphate synthase, which had the greatest variation among the examined activities, was low or undetectable in the first 2 d and showed some increase in d 3 and 4.

The interrelationship among sugars, sorbitol, and enzyme activities in germinating soybean cotyledons and axes during incubation is shown in Table IV. Germination time was highly correlated with the decrease in sucrose content and weakly with the increase in sucrose phosphate synthase activity. The decrease in sucrose content was weakly correlated with the increase in invertase activity and glucose, fructose, and sorbitol content. Sorbitol levels were highly correlated ($P < 0.001$) with the levels of glucose, fructose, and invertase activity, whereas all these compounds and invertase activity had no correlation with the activities of sucrose synthase and sucrose phosphate synthase.

Enzymes of hexose metabolism were assayed in extracts of soybean cotyledons and axes harvested after 2 d of germination to evaluate the possible sources of sorbitol in germinating soybean seeds (Table V). Ketose reductase, which converts

Table II. *Sugar and Sorbitol Content in Cotyledons and Axes of Germinating Soybean Seeds*

Values represent mean of four determinations (± SE) from two separate experiments. HPLC procedures for sugar analysis are described in "Materials and Methods."

Tissue	Days of Germination	Sucrose	Glucose	Fructose	Sorbitol
			mg·g dry weight⁻¹		
Cotyledon	0	55.5 ± 1.2	5.8 ± 1.2	ND[a]	ND
	1	53.6 ± 2.4	5.3 ± 0.4	ND	ND
	2	50.5 ± 2.7	7.8 ± 1.3	ND	ND
	3	34.7 ± 1.6	5.1 ± 0.4	ND	ND
	4	23.3 ± 7.6	5.1 ± 0.7	ND	ND
Axis	0	42.7 ± 4.1	5.0 ± 0.9	ND	ND
	1	55.8 ± 3.0	14.6 ± 1.6	11.0 ± 2.6	6.5 ± 0.8
	2	26.0 ± 3.6	38.9 ± 3.0	51.4 ± 8.8	17.3 ± 3.1
	3	23.6 ± 2.2	34.5 ± 3.5	57.2 ± 9.0	17.7 ± 4.7
	4	19.0 ± 0.5	33.9 ± 1.1	46.4 ± 10.2	16.3 ± 6.1

[a] Not detected.

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Table III. *Activities of Enzymes of Sucrose Metabolism in Germinating Soybean Cotyledons and Axes*

Values are the mean (± SE) of four extractions, two from each separate experiment.

Tissue	Days of Germination	Invertase	Sucrose Synthase	Sucrose Phosphate Synthase
		$\mu mol \cdot min^{-1} \cdot g\ dry\ wt^{-1}$		
Cotyledon	0	0.21 ± 0.04	ND[a]	0.98 ± 0.05
	1	0.23 ± 0.03	ND	0.82 ± 0.05
	2	0.14 ± 0.04	2.12 ± 0.91	1.35 ± 0.58
	3	0.11 ± 0.02	0.44 ± 0.12	0.77 ± 0.10
	4	0.14 ± 0.04	0.63 ± 0.26	1.78 ± 0.26
Axis	0	0.08 ± 0.02	1.81 ± 0.13	ND
	1	1.17 ± 0.03	1.58 ± 0.19	0.75 ± 0.24
	2	5.74 ± 1.40	1.81 ± 0.19	ND
	3	5.81 ± 1.19	0.87 ± 0.21	1.31 ± 0.09
	4	6.72 ± 0.99	1.49 ± 0.49	1.02 ± 0.11

[a] Not detected.

fructose to sorbitol, was present in fair amounts of activity in axes but not detectable in cotyledons. Aldose reductase, which converts glucose to sorbitol, was also present in fair amounts of activity in axes but absent in cotyledons. Both enzymes utilized either NADH or NADPH as cofactors. Both fructokinase and glucokinase activities were present in the cotyledons and axes. Fructokinase activity was higher in axes than in the cotyledons, whereas glucokinase activity was higher in cotyledons than in the axes.

DISCUSSION

Sorbitol has been found in soybean axes and appears to be produced during germination (Fig. 1). Mature 'Williams 82' soybean seeds did not contain sorbitol, nor did the imbibed seeds. The accumulation of sorbitol was found to be highly correlated with the levels of glucose and fructose and the activity of invertase (Table IV). These results suggest the presence of the sorbitol pathway described in mammals and fungi (13). In this pathway, an interconversion of unphosphorylated glucose to fructose (or vice versa), as catalyzed by aldose reductase and sorbitol dehydrogenase, would occur with sorbitol as an intermediate. The finding that germinating soybean axes also contain aldose reductase and ketose reductase (Table V), two catalytic activities needed for the formation of sorbitol from glucose and fructose, is consistent with

the hypothesis that the sorbitol pathway may also function in soybean axes. The fact that cotyledons do not accumulate sorbitol and contain no detectable activities of aldose reductase and ketose reductase further suggests that these enzyme activities are responsible for the presence of sorbitol in germinating soybean axes. The sorbitol pathway was first suggested to exist in developing corn kernels (9) based on the presence of sorbitol and ketose reductase activity. In that study, ketose reductase had been isolated and partially characterized, but aldose reductase was not identified. In the present study, we were unable to purify either the soybean aldose or ketose reductase for further characterization due to the instability of these enzymes. No other reports of a sorbitol pathway in plants have appeared.

The results presented here suggest that the accumulation of sorbitol may play a role in facilitating hexose metabolism. During germination, soybeans convert oil and soluble oligosaccharides, such as raffinose sugars, into sucrose which is utilized by axes for rapid expansion and growth. Prior to utilization, sucrose must be metabolized into simple sugars, a process that may be accomplished by a large increase of invertase activity, as shown in germinating soybean axes (Table III) concomitant with a large increase in glucose and fructose in the tissue (Table II). Additional fructose can also be derived from sucrose by sucrose synthase already present

Table IV. *Correlation Matrix of Characteristics Examined in Germinating Soybean Cotyledons and Axes*

	Days of Germination	Sucrose	Glucose	Fructose	Sorbitol	Invertase	Sucrose Synthase
Sucrose	−0.82**						
Glucose	0.40	−0.63*					
Fructose	0.42	−0.67*	0.99**				
Sorbitol	0.40	−0.63*	0.99**	0.99**			
Invertase	0.47	−0.69*	0.98**	0.98**	0.98**		
Sucrose synthase	0.03	−0.14	0.37	0.29	0.34	0.30	
Sucrose phosphate synthase	0.55*	−0.15	−0.18	−0.12	−0.15	−0.11	−0.32

*, ** Significant at the probability levels of 0.05 and 0.01, respectively.

Table V. *Activities of Enzymes of Hexose Metabolism in Two-Day Germinating Soybean Cotyledons and Axes*

Values are the mean (± se) from five extractions.

Enzyme	Cotyledon	Axis
	$\mu mol \cdot min^{-1} \cdot g\ dry\ wt^{-1}$	
NADH-ketose reductase	ND[a]	0.76 ± 0.14
NADPH-ketose reductase	ND	0.78 ± 0.08
NADH-aldose reductase	ND	0.43 ± 0.06
NADPH-aldose reductase	ND	0.53 ± 0.06
Fructokinase	0.39 ± 0.09	0.79 ± 0.21
Glucokinase	0.63 ± 0.12	0.23 ± 0.09

[a] Not detected.

in the axes. Glucose and fructose would then be converted by hexose kinases to produce hexose phosphates for subsequent metabolism. Since fructokinases from plant sources, such as pea seeds (7, 21), maize kernels (10), and soybean nodules (8) exhibit strong substrate inhibition by fructose, it seems plausible that the presence of sorbitol pathway in axes may provide a modulation function by converting excess fructose into sorbitol and, hence, facilitating the metabolism of free glucose and fructose. It is not clear, however, how the expression of aldose and ketose reductases is controlled in the process, or to what extent the sorbitol pathway functions beyond the germination period examined in this study (4 d). Unlike the axes, cotyledons contained only low levels of invertase activity and did not accumulate hexoses (Tables II and III). Consequently, the sorbitol pathway may not be present in this tissue as demonstrated by the absence of sorbitol accumulation (Table II) and detectable activities of aldose and ketose reductases (Table V).

Several other differences in sucrose and hexose metabolism between the cotyledons and axes of germinating soybean seeds are apparent from the results presented here. Sucrose phosphate synthase activity was consistently higher in cotyledons than in axes, whereas sucrose synthase was higher in axes than in cotyledons (Table III). This is consistent with the idea that sucrose phosphate synthase functions to synthesize sucrose in the cotyledon during germination (3) from the metabolism of seed storage products, such as oil, whereas sucrose synthase functions to breakdown sucrose transported from the cotyledon to the growing axes during germination. It is not clear why glucokinase activity was higher than fructokinase activity in the cotyledons, whereas fructokinase activity was higher than glucokinase activity in the axes.

In conclusion, this report had identified the presence of sorbitol in germinating soybean axes, but not in cotyledons, and it has also demonstrated a strong correlation between sorbitol and the levels of glucose, fructose, and invertase activity. Aldose reductase and ketose reductase activities have been detected in the axes, but not in the cotyledons. The results support the hypothesis that glucose and fructose may be interconverted in germinating soybean axes via the sorbitol pathway (13) with sorbitol as an intermediate. It is predicted that similar systems will be found in many plant species where free glucose and fructose are abundant during seed germination.

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