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Soluble Carbohydrates in Soybean

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1. Introduction

Soybean seeds accumulate soluble carbohydrates throughout development and maturation. Soluble carbohydrates may have important roles in seed germination, seed desiccation tolerance, and cold stress tolerance. The analysis of soluble carbohydrates in soybean seeds and other plant parts at various growth stages (Obendorf et al., 1998b & 2009; Kosina et al., 2009), under environmental stresses (Blackman *et al*., 1992; Buitink et al., 2004; Caffrey et al., 1988; Koster & Leopold, 1988; Obendorf et al., 1997; Rosnoblet et al., 2007; Obendorf et al., 2008b) and in mutant lines (Sebastain et al., 2000; Hitz et al., 2002; Obendorf et al., 2008b & 2009) has provided valuable information about the potential roles of these compounds. The methods for analysis of soluble carbohydrates involve extraction of compounds and quantification by HPLC or high resolution gas chromatography. A special method for measuring transport unloading from the seed coat to the embryo has been developed (Thorne & Rainbird, 1983; Rainbird et al., 1984; Ellis & Spanswick, 1987; Gomes et al., 2005; Kosina et al., 2009 & 2010). In the leaves of soybean plants, glucose is converted to glucose-6-phosphate and then to *myo*-inositol. Maternally, *myo*-inositol is converted to D-pinitol (1D-3-*O*-methyl-*chiro*-inositol) through D-ononitol (1D-4-*O*-methyl-*myo*-inositol) as an intermediate; *myo*-inositol also is converted to D-*chiro*-inositol. Three cyclitols (*myo*-inositol, D-pinitol, and D-*chiro*-inositol) along with sucrose are transported to developing seeds via the phloem where they are unloaded from the seed coat into the apoplastic space surrounding the embryo. During seed maturation the transported free cyclitols accumulate as galactosyl cyclitols, digalactosyl cyclitols, or trigalactosyl cyclitols in the axis and cotyledons of the maturing embryos. Sucrose accumulates as sucrose and as raffinose family oligosaccharides (RFO; raffinose, stachyose and verbascose), *myo*-inositol accumulates as galactinol series oligosaccharides (galactinol, digalactosyl *myo*-inositol and trigalactosyl *myo*-inositol), D-*chiro*-inositol accumulates as fagopyritols (fagopyritol B1, fagopyritol B2, and fagopyritol B3), and D-pinitol accumulates as galactopinitols (galactopinitol A, galactopinitol B, ciceritol, and trigalactopinitol A). In the seed, *myo*-inositol also is converted to phytic acid (*myo*-inositol hexa*kis*phosphate) which is stored in seed protein bodies as phytin (the potassium, sodium, and magnesium salts of phytic acid), a major source of phosphorus and cation chelation. Raffinose family oligosaccharides and phytin can result in reduction of the digestibility and the economic, environmental and dietary value of soybean seed. Consumption of RFO from mature seed products results in flatulence in humans and non-ruminants in addition to reduced digestibility in chickens and pigs (Sebastian et al., 2000). Reducing raffinose and stachyose accumulation in soybean seeds results in increases in metabolizable energy in soybean feed (Sebastian et al., 2000) and reduces flatulence in

humans (Suarez et al., 1999). When consumed, phytic acid is a major inhibitor of both calcium (Heaney et al., 1991) and iron (Lynch et al., 1994) absorption in humans and also results in high phytate concentrations in the manure of chickens and pigs (Sebastian et al., 2000; Hitz et al., 2002). Phosphorus runoff from manure accumulates in lakes and streams resulting in their subsequent eutrophication (Sharpley et al., 2003). In comparison, consumption of products with low phytic acid improves mineral absorption (Heaney et al., 1991; Lynch et al., 1994) and reduces livestock fecal and urinary total phosphorus by 40% with an increase of less damaging and essential nutrient, absorbable (digestible) inorganic phosphorus (Htoo et al., 2007). Mutants have been identified which reduce the accumulation of these undesirable compounds in soybean seeds.

Several useful reviews on soluble carbohydrates in seeds have been published (Dey, 1990; Horbowicz & Obendorf, 1994; Avigad & Dey, 1997; Obendorf, 1997; Loewus & Murthy, 2000; Górecki et al., 2001; Kadlec et al., 2001; Peterbauer & Richter, 2001; Raboy, 2009). This chapter describes the soluble carbohydrate composition of soybean seeds, the structures and biosynthetic pathways, accumulation of soluble carbohydrates during seed development and maturation and their degradation during hydration and germination, a description of changes in soluble carbohydrates in soybean seeds expressing mutant *stc1* and *mips* phenotypes, and the trade-off between improved nutritional quality and agronomic performance of seeds with modified soluble carbohydrate composition.

2. Soluble carbohydrate extraction and analysis

2.1 Greenhouse growth of soybean plants

Locally (42° north latitude) adapted genotypes and cultivars of soybean (maturity groups I and II with indeterminate growth habit) are grown throughout the year in a greenhouse at 27°C day (14 hours) and 21°C night (10 hours) with natural sunlight supplemented 14 hours daily with 740 μmole cm^{-2} $hour^{-1}$ incandescent light from 1000 watt BU Sylvania metal-halide lamps positioned above the plants (Fig. 1). After inoculation of soybean seeds with

Fig. 1. Soybean plant growth in greenhouse.

Bradyrhizobium japonicum, three seeds are placed at 1-cm depth in moist greenhouse soil mix in 4-L pots. The soil mix is composed of equal volumes of silty clay loam soil and artificial medium. The artificial medium contains 0.2 m^3 coarse vermiculite, 0.2 m^3 peat moss, 0.5 kg ferrous sulfate, and 1 kg commercial fertilizer (10-10-10, % as N, P_2O_5, and K_2O equivalents). At 1 week after emergence, seedlings are thinned to 1 plant per pot. Plants are thoroughly watered and fertilized with 2 g pot^{-1} of commercial fertilizer (20-20-20, % as N, P_2O_5, and K_2O equivalents) in water at weekly intervals. Plants are debranched to promote pod set on the main stem, and plants are rotated on the greenhouse bench weekly. This method provides a continuous supply of soybean plants, pods, and seeds at all stages of seed development and maturation for experimentation throughout the year. Pods and seeds may be selected at specific growth stages to synchronize samples for experimentation.

2.2 Soybean stem-leaf-pod explants

Soybean stem-leaf-pod explants which include one internode, one leaf, and one pod with three immature seeds (280–300 mg fresh weight each; about 35 days after pollination; at mid-seed fill before accumulation of RFO, fagopyritols, and galactopinitols) are prepared for feeding exogenous substrates (Gomes et al., 2005; Obendorf et al., 2008a; Kosina et al., 2010) and analysis of seed coat unloading (Kosina et al., 2010). The cut, basal end of the internode (stem) of each explant is placed in a 125-mL Erlenmeyer flask (one explant per flask) containing 100 mL of a feeding solution (Fig. 2). Each solution is loaded into an explant through the cut stem and transported to the leaf by the transpiration stream and to the seed coat through the phloem (Fig. 2). The effect of feeding specific substrates on the composition of mature dry seeds can be determined after feeding the explants for 1-2 weeks followed by slow drying of the explants to facilitate maturation of the seeds (Gomes et al., 2005; Obendorf et al., 2008a).

Fig. 2. Soybean stem-leaf-pod explants in cyclitol solutions or a control solution.

2.3 Soybean seed coat cup

The use of seed coat cups (Fig. 3), formed by surgically removing the immature embryo from the immature soybean seed forming an empty seed coat, is a useful technique to study compounds unloaded from the seed coat into the apoplastic space surrounding the embryo

(Thorne & Rainbird, 1983; Rainbird et al., 1984; Ellis & Spanswick, 1987; Gomes et al., 2005; Kosina et al., 2009 & 2010). Seed coat cup unloading analysis is performed on the middle seed using the surgical method of removing the distal half of the seed coat and the entire embryo from the intact seed coat cup (Fig. 3; Thorne & Rainbird, 1983; Ellis & Spanswick, 1987; Gomes et al., 2005; Kosina et al., 2009 & 2010). Because buffer, salts and mannitol (Thorne & Rainbird, 1983) interfere with derivatization of soluble carbohydrates for analysis by gas chromatography, unloaded compounds are collected in water (Gomes et al., 2005; Kosina et al., 2009 & 2010). Freshly prepared, empty seed coat cups are rinsed two times with distilled water to remove residues and fragments left over from the excision process (Ellis & Spanswick, 1987). The seed coat cup is filled with 200 µL ddH_20 and four 200-µL samples are collected at 30-min intervals for 2 hours (cups refilled after each sampling). An equal volume of ethanol and a known amount of internal standard are added to the sample which is dried and derivatized for analysis by gas chromatography.

Sucrose unloading rates into surgically prepared seed coat cups are comparable to the sucrose unloading rates in plants that are not surgically altered (0.5-1.0 µmole h^{-1}; Thorne & Rainbird, 1983). We have successfully used the seed coat cup method to identify the soluble carbohydrates unloaded by seed coats on intact soybean plants (Gomes et al., 2005; Kosina et al., 2009) and also by seed coats on soybean stem-leaf-pod explants after feeding specific substrates to explants (Kosina et al., 2010).

Fig. 3. Soybean seed coat cup on plant. Seed coat cups are filled with distilled water. Samples are taken at 30-minute intervals for 2 hours and analyzed for soluble carbohydrates unloaded from seed coats.

2.4 Extraction of water-soluble carbohydrates

Soybean, other oil seeds or seed parts (one axis, one or two cotyledons, one seed coat) may be finely pulverized by placing seeds or seed parts in liquid nitrogen and grinding the frozen tissues to a fine powder with a mortar and pestle that is pre-chilled with liquid nitrogen. Tissue (3 to 300 mg) pulverization is easily performed with frozen immature seeds or frozen mature dry seeds or seed parts. A single seed, axis, cotyledon, or seed coat may be prepared for extraction and analysis. Soluble carbohydrates may be extracted in water or hot water. Unfortunately, water extracts may also include contaminating proteins, hydrolytic enzymes, and sometimes cell wall or membrane components. Extraction with aqueous alcohol (water:ethanol, 1:1, v/v) minimizes contamination and activity of hydrolytic enzymes. Passing the aqueous ethanol extract through a 10,000 molecular weight cut-off filter can remove many of the contaminating protein components. Heating the aqueous

ethanol extract to 80°C may inactivate hydrolytic enzymes and minimize degradation of oligosaccharides and galactosyl cyclitols. Heating acidic plant tissue extracts may result in specific artifacts. For example, glutamine readily cyclizes to pyrrolidone carboxylic acid at 100°C (Chibnall & Westall, 1932). *myo*-Inositol may undergo chemical isomerization after heating under specific conditions (Sasaki et al., 1988; Taguchi et al., 1997). Therefore, seed culture media are sterilized by ultafiltration (Saab & Obendorf, 1989; Obendorf et al., 1990; 1998a; 1998b; Wettlaufer & Obendorf, 1991). The filtrate of the aqueous ethanolic extracts of seeds or seed parts may be evaporated under a stream of nitrogen gas at room temperature leaving a dry residue for liquid chromatography or for derivatization in preparation for analysis by gas chromatography. Larger volumes may be freeze-dried when extracting, concentrating, and purifying standards from plant materials.

Typically, the frozen powder from one soybean axis is homogenized with 0.6 mL of ethanol:water (1:1, v/v) containing 100 µg of phenyl α-D-glucoside as internal standard in a ground-glass tissue homogenizer, one soybean cotyledon is extracted in 2.0 mL of ethanol:water (1:1, v/v) containing 300 µg of phenyl α-D-glucoside as internal standard in a ground-glass tissue homogenizer, and one soybean seed coat is extracted in 1.0 mL of ethanol:water (1:1, v/v) containing 100 µg of phenyl α-D-glucoside as internal standard in a ground-glass tissue homogenizer. The extracts are centrifuged at 15,000 x g in a microfuge. Aliquots (500 µL) of the cleared supernatants are passed through a 10,000 molecular weight cut-off filter (Nanosep 10K Omega, Pall Life Sciences, Ann Arbor, Michigan, USA), 200 µL of each filtrate is dried under a stream of nitrogen gas and stored over P_2O_5 overnight to remove traces of water. The dried residues are derivatized with trimethylsilyl-

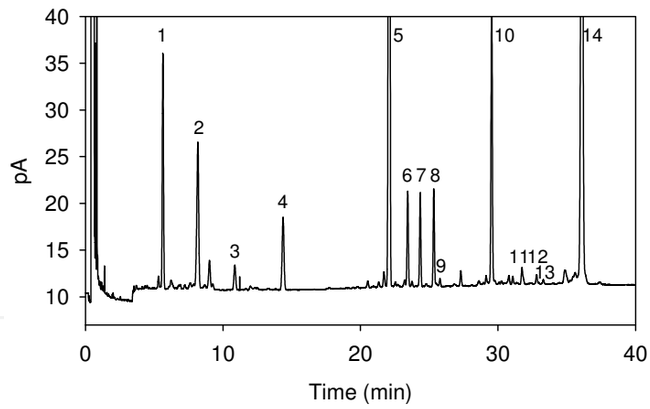

Fig. 4. Gas chromatogram of mature soybean seed cotyledon extract. Dried residues of extracts including the internal standard phenyl α-D-glucoside were derivatized with trimethylsilylimidazole (TMSI):pyridine and analyzed by gas chromatography (Horbowicz & Obendorf, 1994) with minor changes (Gomes et al., 2005) on an HP-1MS capillary column (15 m length, 0.25 mm internal diameter, 0.25 µm film thickness). Identification of peaks: D-pinitol (**1**), D-*chiro*-inositol (**2**), *myo*-inositol (**3**), phenyl α-D-glucoside (internal standard) (**4**), sucrose (**5**), galactopinitol A (**6**), galactopintiol B (**7**), fagopyritol B1 (**8**), galactinol (**9**), raffinose (**10**), ciceritol (**11**), fagopyritol B2 (**12**), DGMI (digalactosyl *myo*-inositol) (**13**), and stachyose (**14**). Soybean seeds expressing the mutant *stc1* phenotype may accumulate small amounts of trigalactosyl cyclitols (not shown in illustration) including trigalactosyl pinitol A (TGPA, 37.8 min), fagopyritol B3 (39.9 min), and trigalactosyl *myo*-inositol (TGMI, 40.4 min).

imidazole:pyridine (1:1, v/v) for analysis by high resolution gas chromatography (Fig. 4). We use silanized glass inserts for drying and derivatization in preparation for analysis by gas chromatography to reduce the potential for chemical isomerization.

2.5 Analysis of soluble carbohydrates

High resolution gas chromatography is the preferred method of analysis of soybean soluble carbohydrates which are relatively small oligomers (monomers to tetramers). Fifteen to thirty different soluble carbohydrates may be identified with good resolution on a single chromatogram (Fig. 4). We use long-cup laminar cup splitter liners (Catalog #20802, Restek International, intltechsupp@restek.com) in the split injection port to facilitate volatilization of the high molecular weight trimethylsilylated carbohydrates (di- and trigalactosides). Some researchers prefer to use direct on-column injection (Traitler et al., 1984).

High pressure liquid chromatography (HPLC) may be preferable for separation of larger oligosaccharides (larger than verbascose, a pentamer) but resolution of monosaccharides and separation of different cyclitols and different galactosyl cyclitols are sometimes problematic when using HPLC.

Analysis of soluble carbohydrates by gas chromatography (Horbowicz & Obendorf, 1994) requires that pure authentic compounds be used as reference standards. Fortunately, many soluble carbohydrates found in soybean seeds are commercially available (see Kadlec et al., 2001, for a listing of sources). Some of the galactosyl cyclitols are not available commercially and must be extracted from plant sources. Kadlec et al. (2001) itemize several plant sources from which standard cyclitols and galactosyl cyclitols may be isolated and provide detailed comparisons of commonly used methods of analysis. Some useful references for the preparation of cyclitols, galactopinitols, fagopyritols, and galactosyl *myo*-inositols include: Ford, 1985; Schweizer et al., 1978; Quemener & Brillouet, 1983; Schweizer & Horman, 1981; Nicolas et al., 1984; Gantner et al., 1991; Horbowicz & Obendorf, 1994; Horbowicz et al., 1998; Szczecinski et al., 1998 & 2000; Obendorf et al., 2000; Steadman et al., 2001; Streeter, 2001; Frank et al., 2009). Recently, the structures of fagopyritol B3, digalactosyl *myo*-inositol (DGMI) and trigalactosyl *myo*-inositol from buckwheat seeds have been confirmed by NMR (Gui, W., Lemley, B.A., Keresztes, I., Condo, A., Steadman, K.J. & Obendorf, R.L., unpublished).

3. Soluble carbohydrate composition of mature seeds

Mature dry soybean seeds may contain 15 to 20 different soluble carbohydrates amounting to approximately 15 to 25% of dry weight (Table 1). Raffinose family oligosaccharides, predominantly stachyose in mature dry soybean seeds, are α-galactosyl derivatives of sucrose (Fig. 5). Sucrose and RFO are the major soluble carbohydrates in soybean seeds (Amuti & Pollard, 1977; Kuo et al., 1988; Horbowicz & Obendorf, 1994; Obendorf et al., 1998b). Other soluble carbohydrates include α-galactosyl derivatives of the cyclitols *myo*- inositol (galactinol and sometimes digalactosyl *myo*-inositol and trigalactosyl *myo*-inositol) (Fig. 6), D-pinitol (galactopinitol A and sometimes digalactosyl pinitol A (ciceritol) and trigalactosyl pinitol A (Fig. 7), and galactopinitol B (Fig. 8), but the digalactosyl pinitol B and trigalactosyl pinitol B oligomers have not been detected in soybean seeds), and D-*chiro*-inositol (fagopyritol B1 and sometimes the di- and tri- galactosyl oligomers fagopyritol B2 and fagopyritol B3) (Fig. 9). Small amounts of *myo*-inositol, D-pinitol, and D-*chiro*-inositol may also be detected in mature

dry seeds. Other than small amounts of maltose (Table 1), reducing sugars are in low concentrations, or not detected, in mature dry seeds. Stachyose and other RFO are not digested by humans, chickens, pigs, and other non-ruminant animals but are microbially fermented in the lower gut resulting in flatulence and reduced feed efficiency (Gitzelmann & Auricchio, 1965; Rutloff et al., 1967; Price et al., 1988; Sebastian et al., 2000).

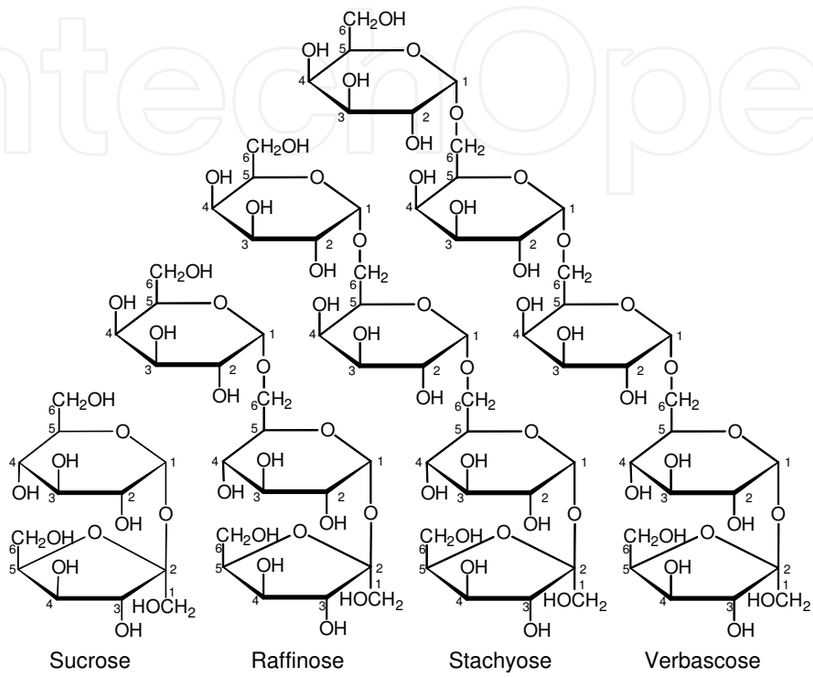

Fig. 5. Raffinose family oligosaccharides (RFO; raffinose, stachyose, and verbascose) are mono-, di- and tri-galactosyl derivatives of sucrose. Sucrose and stachyose are the major soluble carbohydrates in mature soybean seeds.

4. Synthesis of soluble carbohydrates

The enzyme hexokinase (EC 2.7.1.1) converts glucose to glucose-6-phosphate. The enzymes *myo*-inositol-phosphate synthase (MIPS; EC 5.5.1.4) and *myo*-inositol-phosphate monophosphatase (IMP; EC 3.1.3.25) convert glucose-6-phosphate to *myo*-inositol (Fig. 10). 1D-*myo*-Inositol-3-phosphate, the name preferred by biochemists, and 1L-*myo*-inositol-1-phosphate, the name preferred by chemists, are the same structure. Of the four *Mips* genes (*Mips1*, *Mips2*, *Mips3*, *Mips4*) identified in soybean, *Mips1* is highly expressed in immature seeds (Hegeman et al., 2001; Hitz et al., 2002; Nunes et al., 2006; Chiera & Grabau, 2007), especially in cotyledons (Hitz et al., 2002; Chappell et al., 2006). *Mips2*, *Mips3* and *Mips4* are poorly expressed in immature seeds; by contrast *Mips4* is highly expressed in leaves (Chappell et al., 2006).

The free cyclitols *myo*-inositol, D-ononitol, D-pinitol, and D-chiro-inositol (Fig. 11) are present in soybean leaves (Streeter, 2001; Streeter *et al*., 2001). D-Ononitol is an intermediate in the conversion of *myo*-inositol to D-pinitol in leaves of legumes (Dittrich & Brandl, 1987). The

enzyme *myo*-inositol *O*-methyl transferase (IMT; EC 2.1.1.129) converts *myo*-inositol to D-ononitol (Vernon & Bohnert, 1992; Vernon et al., 1993; Wanek et al., 1995). The enzyme(s) for conversion of D-ononitol to D-pinitol is unknown but is believed to be a two-step oxidoreductase with an inosose as an intermediate (Fig. 11; Obendorf, 1997). Likewise, the

Soluble carbohydrate	Soybean Line			
	CHECK	LRS	LRSP1	LRSP2
	mg (g dry weight)$^{-1}$ ± SE			
Sucrose	57.22 b	84.84 b	212.12 a	91.96 b
Raffinose	16.70 a	0.82 c	5.58 b	6.65 b
Stachyose	133.60 a	6.44 b	3.27 b	3.28 b
Verbascose	6.90 a	1.60 b	0 c	0 c
myo-Inositol	0.60 ab	1.77 a	1.20 a	0.03 b
Galactinol	0.55 b	8.93 a	0 b	0.01 b
Digalactosyl *myo*-inositol	0.34 b	8.38 a	0 b	0.03 b
Trigalactosyl *myo*-inositol	0 b	0.43 a	0 b	0 b
D-Pinitol	6.67 b	0.60 c	28.63 a	10.36 b
Galactopinitol A	7.39 a	9.34 a	1.20 b	1.29 b
Galactopinitol B	5.47 a	3.15 b	1.14 c	0.91 c
Ciceritol	2.00 b	9.17 a	0.03 c	0.10 c
Trigalactosyl pinitol A	0 b	1.22 a	0 b	0 b
D-*chiro*-Inositol	0.40 b	0.06 b	1.47 a	0.16 b
Fagopyritol B1	4.57 a	2.59 b	2.10 b	2.14 b
Fagopyritol B2	0.69 a	1.33 a	0.05 b	0.09 b
Fagopyritol B3	0 b	0.27 a	0 b	0 b
Fructose	0.62 b	0.56 b	5.76 a	0.27 b
Glucose	0.33 b	0.41 b	1.75 a	0.25 b
Maltose	2.75 a	5.27 a	5.36 a	5.48 a
Total soluble carbohydrates	246.87 ab	147.18 bc	269.63 a	123.98 c
Total RFO	157.21 a	8.86 b	8.85 b	9.92 b
Total α-galactosides	178.28 a	53.66 b	13.36 c	14.49 c
Ratio (sucrose:RFO)	0.51 c	11.24 b	36.46 a	10.36 b
Ratio (sucrose:α-galactosides)	0.43 c	1.72 c	20.07 a	6.83 b

Table 1. Soluble carbohydrates in cotyledon tissues from seeds of four soybean lines (from Obendorf et al., 2008b). For comparisons between soybean lines, means not connected by the same letter are significantly different ($P < 0.05$) after a Tukey correction for multiple comparisons. RFO = raffinose family oligosaccharides (raffinose + stachyose + verbascose).

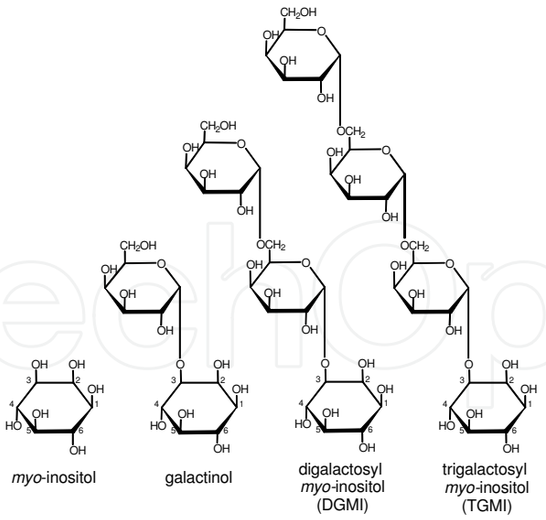

Fig. 6. Galactinol series. Small amounts of galactinol (α-D-galactopyranosyl-(1→3)-1D-*myo*-inositol or α-D-galactopyranosyl-(1→1)-1L-*myo*-inositol) and sometimes digalactosyl *myo*-inositol are detected in soybean seeds. Seeds with low RFO accumulation or with elevated galactinol also may have increased amounts of digalactosyl *myo*-inositol and detectable amounts of trigalactosyl *myo*-inositol.

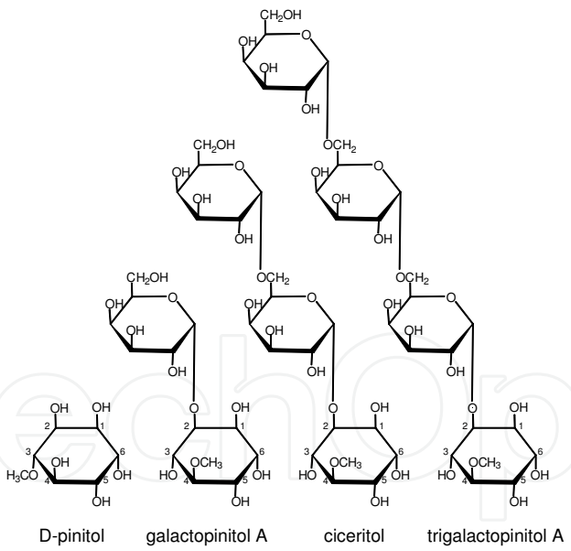

Fig. 7. Galactopinitol A series. Normal soybean seeds accumulate mostly galactopinitol A (α-D-galactopyranosyl-(1→2)-1D-4-*O*-methyl-*chiro*-inositol) and small amounts of ciceritol (digalactosyl pinitol A). Soybean seeds expressing the *stc1* mutant, with low raffinose synthase activity and low accumulation of stachyose, may accumulate small amounts of trigalactosyl pinitol A. In the galactopinitol A series, the galactosyl residues attach to the 5-position of D-pinitol; upon attachment, the carbons are renumbered: 6 becomes 1, 5 becomes 2, and 3 becomes 4.

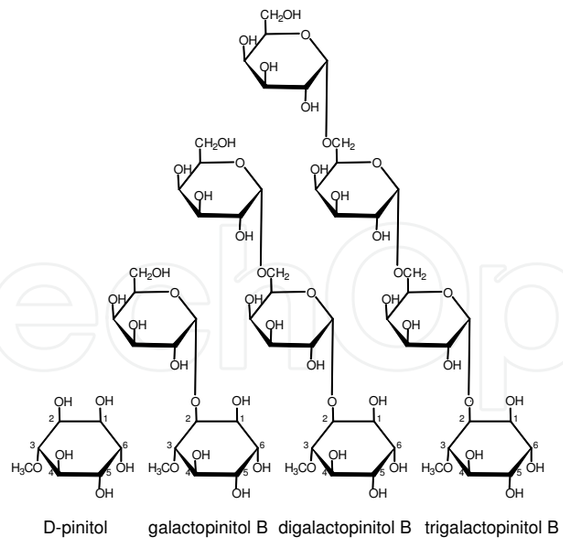

Fig. 8. Galactopinitol B series. Only galactopinitol B (α-D-galactopyranosyl-(1→2)-1D-3-*O*-methyl-*chiro*-inositol) accumulates in soybean seeds. Digalactosyl pinitol B and trigalactosyl pinitol B have not been detected in soybean. In the galactopinitol B series, the galactosyl residues attach to the 2-position of D-pinitol.

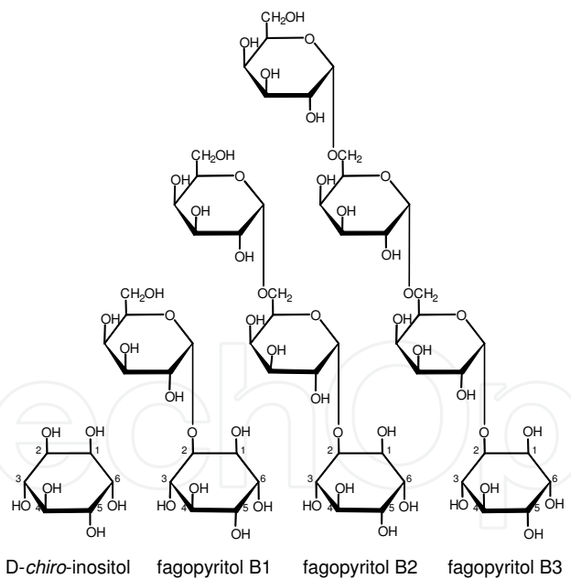

Fig. 9. Fagopyritol B series. Fagopyritol B1 (α-D-galactopyranosyl-(1→2)-1D-*chiro*-inositol) is the dominant fagopyritol in normal mature soybean seeds. Seeds fed free D-*chiro*-inositol or seeds expressing the mutant *stc1* phenotype with low RFO accumulation also may have fagopyritol B2 and small amounts of fagopyritol B3. Only the fagopyritol B series compounds are detected in mature soybean seeds. Fagopyritol A series compounds, (fagopyritol A1, α-D-galactopyranosyl-(1→3)-1D-*chiro*-inositol) have not been detected in soybean.

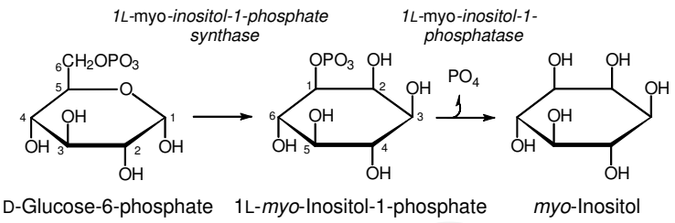

Fig. 10. Synthesis of *myo*-inositol. The structures 1D-*myo*-inositol-3-phosphate and 1L-*myo*-inositol-1-phosphate are the same structure.

enzyme(s) for conversion of *myo*-inositol to D-*chiro*-inositol is unknown but is believed to be a two-step oxidoreductase with an inosose as an intermediate (Fig. 11). There is no evidence to support the proposed synthesis of D-*chiro*-inositol from D-pinitol in soybean (Obendorf, 1997; Obendorf et al., 2004). Surgically removing the immature embryo from immature soybean seed to form an empty seed coat cup has been used to study compounds unloaded from the seed coat into the apoplastic space surrounding the embryo (Thorne & Rainbird, 1983; Rainbird et al., 1984; Ellis & Spanswick, 1987; Gomes et al., 2005; Kosina et al., 2009 & 2010). Sucrose (90% of C), amides (glutamine, 52% of N; asparagine, 19% of N) and amino acids are the most abundant compounds unloaded by soybean seed coats (Rainbird et al., 1984; Ellis & Spanswick, 1987).

Additionally, *myo*-inositol, D-pinitol and D-*chiro*-inositol are transported to the seed and unloaded from the seed coat into the apoplastic space surrounding the developing embryo (Gomes et al., 2005; Kosina et al., 2009 & 2010). The reducing sugars glucose, fructose, and maltose are detected in variable but small amounts.

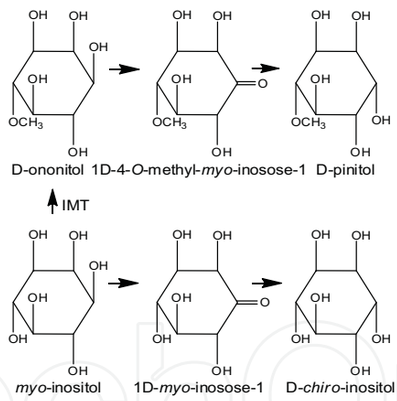

Fig. 11. Soybean cyclitols. Soybean leaves have four cyclitols: *myo*-inositol, D-ononitol (1D-4-*O*-methyl-*myo*-inositol), D-pinitol (1D-3-*O*-methyl-*chiro*-inositol) and D-*chiro*-inositol. *myo*-Inositol is converted to D-pinitol through D-ononitol as an intermediate in legume leaves (Dittrich and Brandl, 1987). The enzyme *myo*-inositol *O*-methyl transferase (IMT; EC 2.1.1.129) converts *myo*-inositol to D-ononitol. The enzyme(s) for conversion of D-ononitol to D-pinitol is unknown but is believed to be a two-step oxidoreductase with an inosose as an intermediate. The enzyme(s) for conversion of *myo*-inositol to D-*chiro*-inositol is unknown but is believed to be a two-step oxidoreductase with an inosose as an intermediate. There is no evidence to support the proposed synthesis of D-*chiro*-inositol from D-pinitol in soybean. *myo*-Inositol, D-pinitol and D-*chiro*-inositol are transported to the seed and unloaded from the seed coat into the apoplastic space surrounding the developing embryo. *myo*-Inositol also may be synthesized in the embryo during seed development.

D-Ononitol, galactinol, galactopinitols, fagopyritols, raffinose, stachyose, and verbascose are not detected in seed coat cup exudates (Gomes et al., 2005; Kosina et al., 2009 & 2010). Seed coat cup unloading rates for D-*chiro*-inositol, *myo*-inositol, D-pinitol, and sucrose average 5.1, 3.6, 32.9, and 147.7 μg hour^{-1}, respectively, on soybean plants (Kosina et al., 2009). *myo*-Inositol also may be synthesized in the embryo during soybean seed development because *Mips* (wild-type *Mips* sequence designation GM mI 1-PS-1A, GenBank accession number AY038802) is expressed in immature cotyledons (Hitz et al., 2002; Chappell et al., 2006). Synthesis of D-pinitol and D-*chiro*-inositol have not been reported in normal soybean embryos. D-Ononitol, the intermediate in the conversion of *myo*-inositol to D-pinitol (Fig. 11) is not detected in soybean seed coat exudates (Gomes et al., 2005; Kosina et al., 2009 & 2010), in soybean embryos (Horbowicz & Obendorf, 1994), or in non-transgenic somatic embryos (Chiera et al., 2006). Transgenic somatic embryos of soybean containing the myo-*inositol* O-*methyl transferase* (*IMT*) gene from *Mesembryanthmum crystallinum* led to an increase in D-ononitol in embryos, compared to non-transgenic embryos, and an increase in D-pinitol in maturing embryos (Chiera et al., 2006).

Sucrose is transported from leaves to seeds, unloaded by seed coats to the apoplastic space surrounding the embryo, and taken up by immature soybean embryos as the major carbon source for seed growth. Raffinose synthase (RFS, EC 2.4.1.82) transfers a galactosyl residue from galactinol (the galactosyl donor) to sucrose (the galactosyl receptor) to form raffinose (Fig. 12). Stachyose synthase (STS, EC 2.4.1.67) transfers a galactosyl residue from galactinol to raffinose to form stachyose (Fig. 12), the most abundant RFO in normal soybean seeds (Table 1) (Avigad & Dey, 1997; Peterbauer & Richter, 2001). Verbascose synthase (VBS) transfers a galactosyl residue from galactinol to stachyose to form verbascose (Fig. 12), usually in small amounts in soybean seeds.

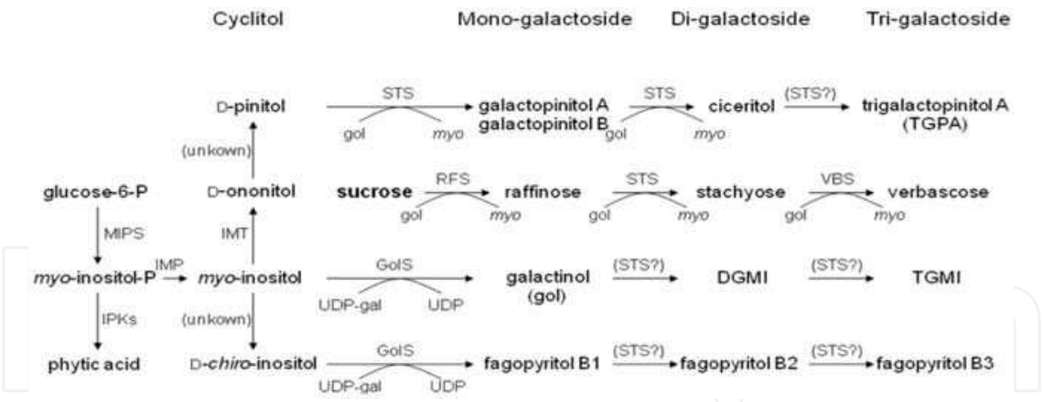

Fig. 12. Proposed pathways for synthesis of cyclitols, cyclitol galactosides, and raffinose family oligosaccharides (from Obendorf et al., 2009). Parentheses (unknown) by an arrow indicates that an enzyme catalyzing the reaction has not been identified. Some reactions may be reversible. DGMI, digalactosyl *myo*-inositol; TGMI, trigalactosyl *myo*-inositol; gol, galactinol; GolS, galactinol synthase (EC 2.4.1.123); IMP, *myo*-inositol-phosphate monophosphatase (EC 3.1.3.25); IPKs, *myo*-inositol-phosphate kinases; MIPS, *myo*-inositol-phosphate synthase (EC 5.5.1.4); *myo*, *myo*-inositol; RFS, raffinose synthase (EC 2.4.1.82); STS, stachyose synthase (EC 2.4.1.67), (STS?) indicates STS, or a similar enzyme, is proposed by extrapolation but has not been demonstrated experimentally; UDP, uridine diphosphate; UDP-gal, uridine diphosphate galactoside; VBS, verbascose synthase.

Maternally synthesized *myo*-inositol may be transported to the soybean seed and unloaded by the seed coat into the apoplastic space surrounding the immature embryo (Gomes et al., 2005; Kosina et al., 2009 & 2010). *myo*-Inositol also may be synthesized in the embryo tissues since *Mips* is expressed in embryos (Hitz et al., 2002; Chappell et al., 2006). Galactinol synthase (GolS, EC 2.4.1.123) transfers a galactosyl residue from UDP-galactose (the galactose donor) to *myo*-inositol (the galactosyl acceptor) to form galactinol (α-D-galactopyranosyl-(1→3)-1D-*myo*-inositol or α-D-galactopyranosyl-(1→1)-1L-*myo*-inositol) (Fig. 12; Obendorf et al., 2004). High availability of *myo*-inositol in embryos leads to elevated galactinol which in turn leads to elevated synthesis of RFO (Fig. 12; Karner et al., 2004). Elevated galactinol may also lead to accumulation of its higher oligomers, digalactosyl *myo*-inositol (DGMI) and trigalactosyl *myo*-inositol (TGMI) (Fig. 12). Enzymes responsible for the accumulation of these higher oligomers of galactinol have not been characterized experimentally, but are predicted to be a stachyose synthase or an enzyme similar to stachyose synthase (STS?; Fig. 12). Metabolism of *myo*-inositol is difficult to follow because *myo*-inositol has multiple roles and forms multiple products including galactinol (Fig. 6), other cyclitols (Fig. 11), cell wall components, membrane components and phytic acid (Loewus & Murthy, 2000; Raboy, 2009).

D-Pinitol is synthesised in leaves (Dittrich & Brandl, 1987; Streeter, 2001; Streeter et al., 2001), transported to seeds, and unloaded by soybean seed coats into the apoplastic space surrounding immature embryos (Figs. 11 & 12; Gomes et al., 2005; Kosina et al., 2009 & 2010). In the embryo, stachyose synthase (STS, EC 2.4.1.67) transfers a galactosyl residue from galactinol to D-pinitol to form galactopintol A plus galactopinitol B (Peterbauer & Richter, 2001; Fig. 12), isomeric compounds due to the presence of the *O*-methyl group of pinitol (Figs. 7 & 8). Only galactopintol A forms higher oligomers in soybean seeds (Fig. 12). Stachyose synthase transfers a galactosyl residue from galactinol (or from galactopinitol A) to glactopinitol A to form ciceritol (Fig. 12; Hoch et al., 1999; Peterbauer and Richter, 2001). It is proposed that stachyose synthase or a similar enzyme also transfers a galactosyl residue from galactinol to ciceritol to form trigalactosyl pinitol A (Fig. 12). Digalactosyl pinitol B and trigalactosyl pinitol B have not been detected in soybean.

Like D-pinitol, D-*chiro*-inositol also is transported to seeds and is unloaded by the seed coat into the apoplastic space surrounding the embryo (Gomes et al., 2005; Kosina et al., 2009 & 2010). Galactinol synthase transfers a galactosyl residue from UDP-galactose (the galactosyl donor) to D-*chiro*-inositol (the galactosyl acceptor) to form fagopyritol B1 (α-D-galactopyranosyl-(1→2)-1D-*chiro*-inositol) (Fig. 12; Obendorf et al., 2004). Soybean galactinol synthase does not form fagopyritol A1 (α-D-galactopyranosyl-(1→3)-1D-*chiro*-inositol) and cannot utilize D-pinitol as a galactosyl receptor to form galactopintols (Obendorf et al., 2004).

5. Modification of soluble carbohydrates in soybean seeds

Seeds of four proprietary soybean [*Glycine max* (L.) Merrill] lines (Table 2) with normal raffinose, stachyose and phytin (CHECK) seeds expressing the normal *Stc1* and *Mips* phenotype; low raffinose and stachyose (LRS) seeds expressing the mutant *stc1* phenotype; low raffinose, stachyose, and phytin (LRSP1, LRSP2) seeds expressing the mutant *mips* phenotype (wild-type *Mips* sequence designation GM mI 1-PS-1A, AY038802; Hitz *et al*., 2002) were provided by Steve Schnebly, Pioneer Hi-Bred, A DuPont Business. All were advanced breeding lines in related, but not isogenic, Group II maturity agronomic backgrounds developed by traditional breeding. The *stc1* and *mips* alleles in these breeding lines are described by Sebastian *et al*. (2000), Hitz *et al*. (2002), and Meis *et al*. (2003).

	CHECK	LRS	LRSP1	LRSP2	References
Raffinose	normal	low	low	low	Sebastian et al., 2000 Hitz et al., 2002
Stachyose	normal	low	low	low	Sebastian et al., 2000 Hitz et al., 2002
Phytic acid	normal	normal	low	low	Sebastian et al., 2000 Hitz et al., 2002
Mutant	*Stc1* normal *Mips* normal	*stc1* mutant *Mips* normal	*Stc1* normal *mips* mutant	*Stc1* normal *mips* mutant	Sebastian et al., 2000 Hitz et al., 2002 Meis et al., 2003
Imbibitional chilling	tolerant	tolerant	sensitive	sensitive	Obendorf et al., 2008b
Field emergence	normal	normal	reduced	reduced	Meis et al., 2003
myo-Inositol-phosphate synthase activity in seeds	normal	normal	low	low	Hitz et al., 2002
Raffinose synthase activity in seeds	normal	low	normal	normal	Hitz et al., 2002
Stachyose synthase activity in seeds	normal	normal	normal	normal	Hitz et al., 2002
Galactinol synthase activity in seeds	normal	normal	normal	normal	Hitz et al., 2002
Galactinol	normal	high	low	low	Sebastian et al., 2000 Hitz et al., 2002
RFO	normal	low	low	low	Sebastian et al., 2000 Hitz et al., 2002
Galactopinitols	normal	higher	lower	lower	Obendorf et al., 2008b
Fagopryitol B1	normal	normal	normal	normal	Obendorf et al., 2008b
Fagopyritols B2 + B3	low	increased	low	low	Obendorf et al., 2008b
Trigalactosyl cyclitols	very low	increased	very low	very low	Obendorf et al., 2009
Feeding D-*chiro*-inositol increased fagopyritol B1	yes	yes	yes	yes	Obendorf et al., 2008a
Feeding *myo*-inositol increased RFO	no	no	yes	yes	Obendorf et al., unpublished Hitz et al., 2002

Table 2. Soybean seed phenotypes of four breeding lines.

Soybean seeds with low raffinose and low stachyose (LRS phenotype) expressing a mutant *stc1* gene conferring reduced raffinose synthase (RFS) activity but normal stachyose synthase (STS) and galactinol synthase (GolS) activities (Sebastian et al., 2000; Hitz et al., 2002) have field emergence and yield comparable to seeds with normal raffinose and stachyose (Neus *et al*., 2005) (Table 2). The low raffinose and stachyose (LRS) phenotype is associated with a novel raffinose synthase allele, RS2 (Dierking & Bilyeu, 2008). LRS seeds expressing the mutant *stc1* phenotype have increased accumulation of galactosyl cyclitols (fagopyritols and galactopintitols) (Obendorf *et al*., 2008b, 2009) and are tolerant to imbibitional chilling (Obendorf *et al*., 2008b) (Table 2). Seeds with low raffinose, stachyose and phytin (LRSP phenotype with 50% less phytin than the normal *Mips* phenotype) expressing a mutant *mips* gene conferring reduced *myo*-inositol-phosphate synthase (MIPS) activity (Sebastian *et al*., 2000; Hitz *et al*., 2002) have decreased field emergence, especially when seeds are produced in subtropical environments (Meis *et al*., 2003), and also are sensitive to imbibitional chilling (Obendorf *et al*., 2008b) (Table 2). Seeds expressing the mutant *mips* phenotype (wild-type *Mips* sequence designation GM mI 1-PS-1A, AY038802; Hitz et al., 2002) with low stachyose and phytin (LRSP1, LRSP2) accumulate very small amounts of galactosyl cyclitols (galactinol, galactopinitols, fagopyritol B2, fagopyritol B3) (Obendorf *et al*., 2008b, 2009), but these seeds can accumulate galactinol, raffinose and stachyose after incubation with *myo*-inositol (Hitz *et al*., 2002) (Table 2). Seeds and isolated embryos of all four lines accumulate fagopyritol B1 after incubation with D-*chiro*-inositol followed by slow drying (Obendorf et al., 2008a; Obendorf, R.L., Sensenig, E.M., Byrt, E.M., Owczarczyk, A.B., Ohashi, M., & Schnebly, S.R., unpublished).

6. Loss of soluble carbohydrates during seed germination

Normal, mature, dry soybean seeds have a high concentration of stachyose (Fig. 13A; Hsu et al., 1973; Amuti & Pollard, 1977; Obendorf et al., 1998b). Upon hydration, stachyose and raffinose concentrations in axis tissues decline to low concentrations before germination (radicle emergence) at 18 hours, followed by an increase in reducing sugars (monosaccharides) (Hsu et al., 1973; Koster & Leopold, 1988). The loss of stachyose in axis tissues (Fig. 13A) correlates with the loss of desiccation tolerance when measured as the rate of leakage from axes after imbibition, desiccation, and rehydration, and emergence of radicles (germination) and shoots following various durations of pre-imbibition (Fig. 13B) (Koster & Leopold, 1988). Loss of non-reducing sugars (raffinose, stachyose, sucrose) is associated with a transient accumulation of starch in axis and cotyledon tissues of soybean seeds (Von Ohlen, 1931; Adams et al., 1980) and with the onset of isocitrate lyase (ICL, EC 4.1.3.1) synthesis and subsequent lipid mobilization after germination (Polanowski & Obendorf, 1991). Conversion of sugars to starch during seed hydration facilitates the increase in solute potential (smaller negative values). Solute potential of axis tissues of hydrating seeds increases to about -1.4 MPa before germination (Egli & TeKrony, 1993).

7. Accumulation of soluble carbohydrates during seed development

During the first 21 days after pollination, the soybean pod increases to about 35 mm in length. After 21 days, the seeds increase in size and expand within the pod (Fig. 14, left). Soybean seeds increase in dry weight in a linear response to maximum fresh weight (Fig. 15A), color changes from green to yellow at maximum seed dry weight (physiological

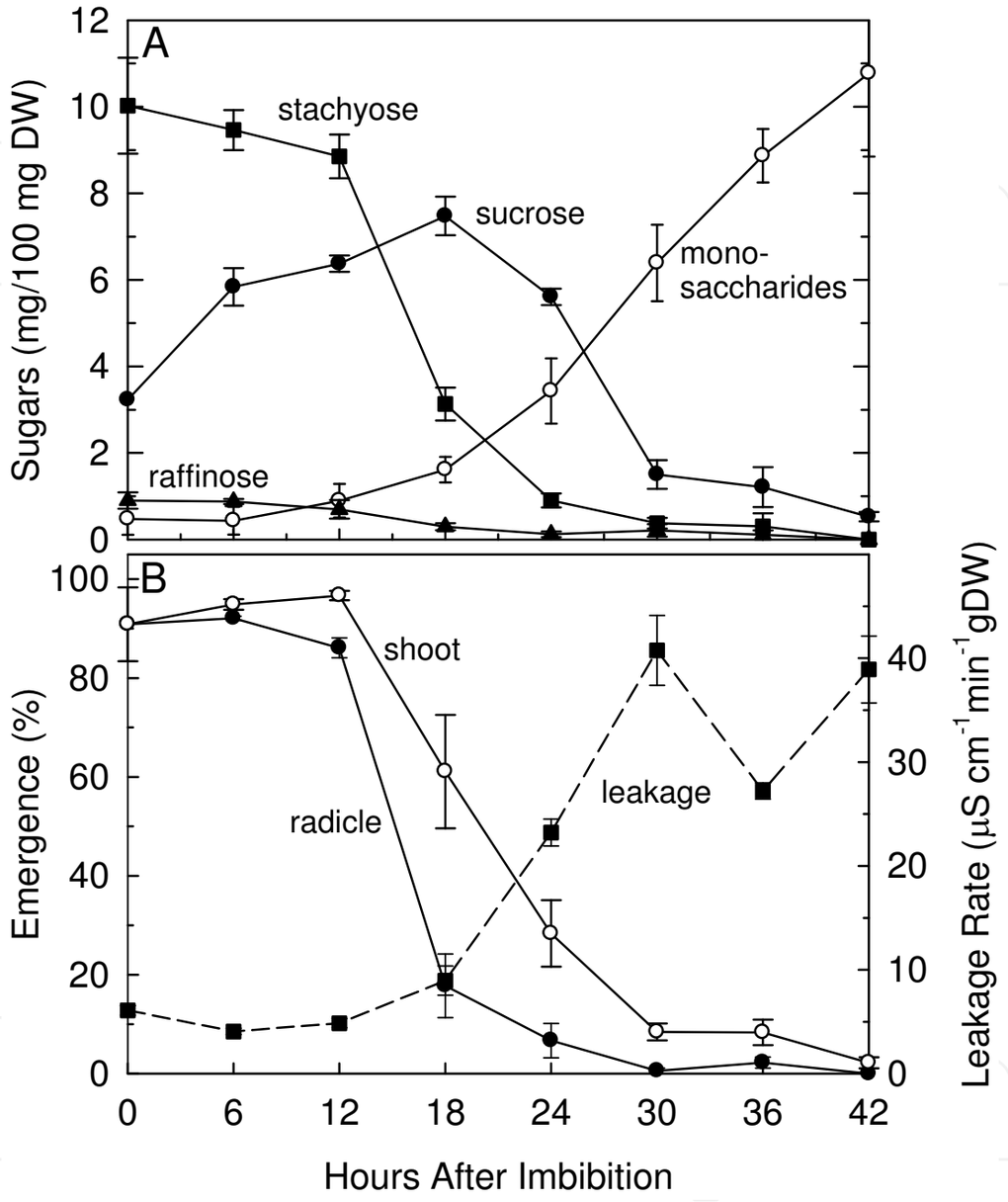

Fig. 13. Changes in soluble sugars and loss of desiccation tolerance during imbibition and post-germination in soybean. A, Changes in sucrose, raffinose, stachyose, and mono-saccharides in axes during imbibition and post-germination of soybean seeds. Radicle emergence (germination) at 18 hours. B, Rate of leakage (squares) from axes after imbibition, desiccation, and rehydration, and emergence of radicles (closed circles) and shoots (open circles) after desiccation following various durations of pre-imbibition (adapted from Koster & Leopold, 1998; this material is copyrighted by the American Society of Plant Biologists and is reprinted with permission).

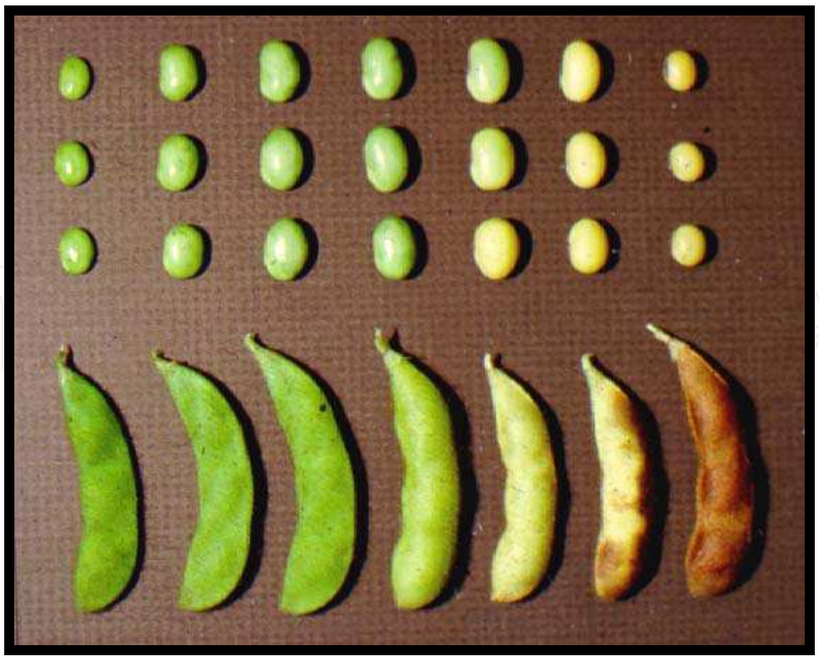

Fig. 14. Soybean seed development and maturation. The number of days after flowering is approximately 24 (left), 34, 38, 48, 54, 58, and 70 (right, mature dry seed).

maturity or mass maturity) (Fig. 15E), and seeds begin to shrink from the pod wall and decrease in size as they begin to lose water during seed desiccation to their mature, dry size (Fig. 14, right). During the linear phase, the soybean seed dry weight growth rate is typically about 5 mg seed^{-1} day^{-1} in the field (Rubel et al., 1972; Obendorf et al., 1980), 8 mg seed^{-1} day^{-1} in the greenhouse (Obendorf et al., 1980), 3-4 mg seed^{-1} day^{-1} in pod culture (Obendorf et al., 1983), and 5-25 mg seed^{-1} day^{-1} in isolated seed culture depending on starting size (Obendorf et al., 1984). Physiological maturity or mass maturity is defined as time of maximum seed dry weight accumulation and represents the cessation of seed dry matter growth. Physiological maturity of individual soybean seeds typically occurs at about 50 days after pollination when seed coat color changes from green to yellow (Fig. 14) and seed moisture is about 60% on a fresh weight basis (Obendorf et al., 1980 & 1998b; Fig. 15). Translocation of sucrose from photosynthate into the embryo ceases when the seed coat changes from green to yellow and seed respiration declines rapidly (TeKrony et al., 1979). Likewise, cotyledons cease to take up sucrose when the cotyledon color changes from green to yellow (Vernooy et al., 1986). At this time, transport of water and nutrients into the seed ceases and the seed begins to shrink as it loses water. Shrinkage of the seed from the pod wall is the most reliable indicator of the cessation of soybean seed growth on plants (Crookston & Hill, 1978). At the time seed growth ceases, the osmotic potential of embryo tissues is about -1.8 MPa (Saab & Obendorf, 1989; Egli, 1990; Slawinska & Obendorf, 1991). Axis tissues turn yellow and cease growth before cotyledons (Obendorf et al., 1984 & 1998b). The pattern of yellowing begins at the radicle tip and progresses up the hypocotyl to the cotyledonary node, whereas cotyledons turn yellow from the edge to the center of each cotyledon (Obendorf et al., 1984 & 1998b). Raffinose and stachyose accumulate late during seed maturation (the yellowing and drying phases; Fig. 15) (Amuti & Pollard, 1977; Yazdi-Samadi et al., 1977; Dornbos & McDonald, 1986; Lowell & Kuo, 1989; Obendorf et al., 1998b

& 2009). About 70% of the RFO, and likewise the galactosyl cyclitols, accumulate after maximum seed dry weight (physiological maturity) during the phase of seed drying (Obendorf et al., 2009). The monogalactosides (raffinose, galactopinitol A, galactopinitol B, fagopyritol B1) start to accumulate when the embryo tissues begin to yellow, followed by the digalactosides (stachyose, ciceritol, fagopyritol B2, digalactosyl *myo*-inositol) and finally the trigalactosides (verbascose, trigalactosyl pinitol A, fagopyritol B3, trigalactosyl *myo*-inositol) during the desiccation phase of seed maturation (Obendorf et al., 2009).

Immature soybean seeds can be precociously matured by slow drying (Adams et al., 1983; Blackman et al., 1992). Germination of immature soybean seeds is similar for seeds undergoing slow drying or for seeds held at high relative humidity to prevent drying (Fig. 16A). Seeds undergoing slow drying develop desiccation tolerance whereas seeds held at high relative humidity do not develop desiccation tolerance (Fig. 16B). Seeds undergoing slow drying accumulate stachyose (Fig. 16D) and are desiccation tolerant (Fig. 16B). In contrast, seeds held at high relative humidity do not accumulate stachyose (Fig. 16D) and do not develop desiccation tolerance (Fig. 16B). Stachyose is not required for germination per se as accumulation of stachyose was not required for germination of seeds held at high relative humidity (Fig. 16A; Blackman et al., 1992). Inhibition of RFO degradation in hydrated soybean seeds did not decrease germination under laboratory conditions with minimal environmental stress, suggesting that RFO metabolism is not an obligatory requirement for soybean germination per se (Dierking & Bilyeu, 2009). These observations do not negate a role of RFO as seed storage reserves. The results merely mean that other readily mobilized reserves are in sufficient supply to meet the needs for germination. Galactinol is the galactosyl donor for the formation of stachyose (Fig. 12). Seeds which are held at high relative humidity do not accumulate stachyose (Fig. 16D) and these seeds accumulate more galactinol (Fig. 16C) than seeds held at high relative humidity. Similarly, LRS seeds expressing the *stc1* mutant have low raffinose synthase activity (Table 2) resulting in lower raffinose, stachyose, and verbascose accumulation but more galactinol than CHECK seeds expressing normal *Stc1* with normal raffinose synthase activity and larger accumulations of raffinose, stachyose, and verbascose (Table 1, Table 2). LRS seeds also accumulate more di- and tri-galactosyl cyclitols than CHECK seeds. LRS seeds are desiccation tolerant, tolerant to imbibitional chilling (Obendorf et al., 2008b), and have normal field emergence (Meis et al., 2003) (Table 2), perhaps because these seeds, that are low in RFO, accumulate more galactosyl cyclitols (Tables 1 & 2). Buckwheat seeds normally accumulate only very small amounts of raffinose and stachyose, but buckwheat seeds do accumulate fagopyritols and are desiccation tolerant with a high germination percentage (Horbowicz & Obendorf, 1994; Horbowicz et al., 1998). It is proposed that fagopyritols and other galactosyl cylitols can function in the same way as stachyose in conveying seed desiccation tolerance and seed performance (Horbowicz & Obendorf, 1994; Horbowicz et al., 1998) in LRS soybean seeds (Obendorf et al., 2008b).

8. Phytic acid

Phytic acid (*myo*-inositolhexa*kis*phosphate) can account for about 75% of the total seed phosphorus and accumulates mostly in seed cotyledon protein bodies as potassium, magnesium, and manganese salts of phytic acid (phytin) (Raboy, 2009). Ingested phytic acid is not efficiently hydrolyzed by humans, chickens, pigs, or other monogastric animals and may contribute to reduced uptake of iron, zinc, and calcium (Heaney et al., 1991; Lynch et

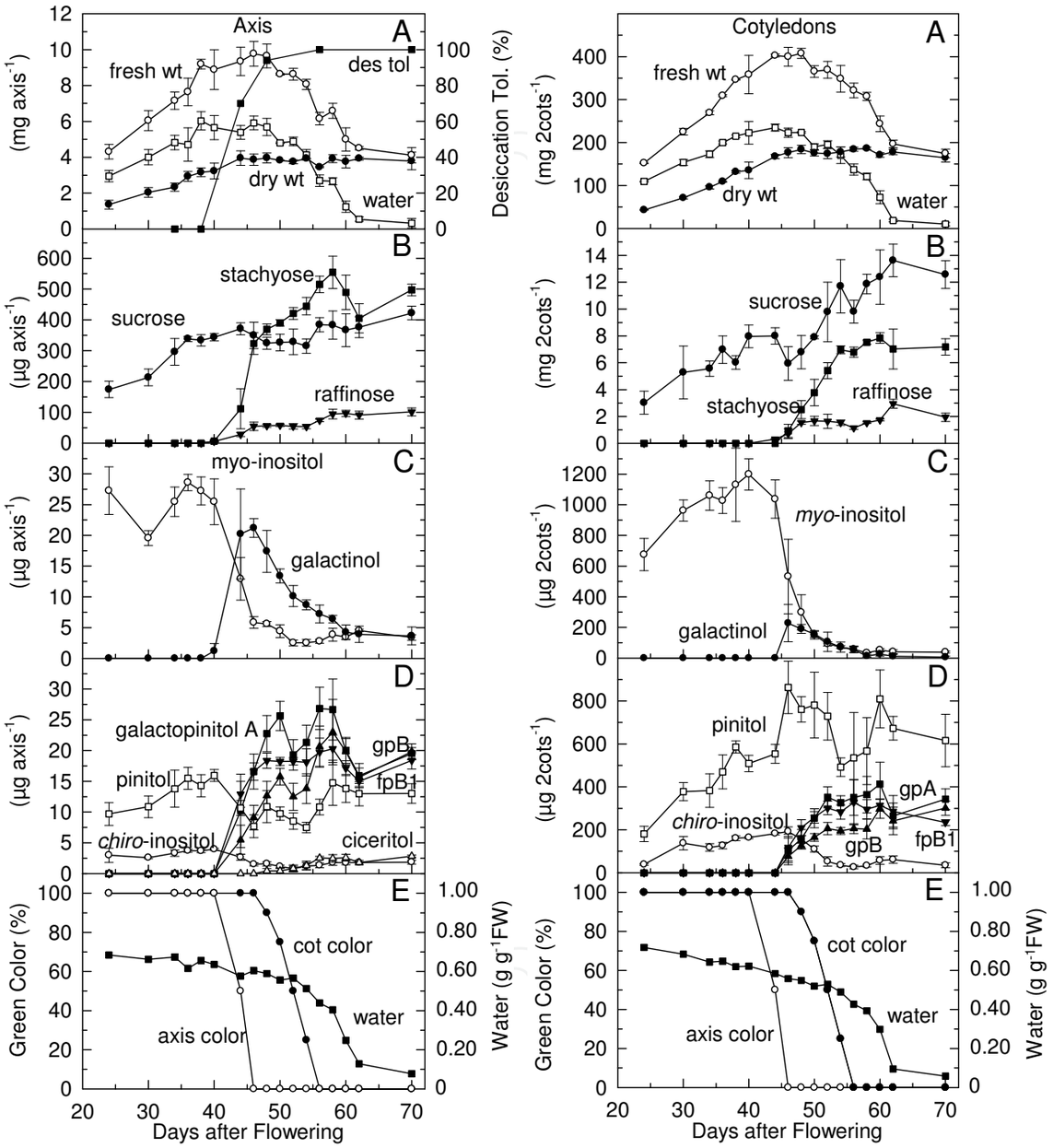

Fig. 15. Soluble carbohydrate accumulation in soybean embryos (axis on left; cotyledons on right) in normal soybean seeds during development and maturation in the greenhouse (from Obendorf et al., 1998b). A, Fresh weight, dry weight, water content, and desiccation tolerance. B, Sucrose, raffinose, and stachyose. C, *myo*-Inositol and galactinol. D, D-Pinitol, galactopinitol A (gpA), galactopinitol B (gpB), ciceritol, D-*chiro*-inositol and fagopyritol B1 (fpB1). E, loss of axis green color, cotyledon green color, and water concentration.

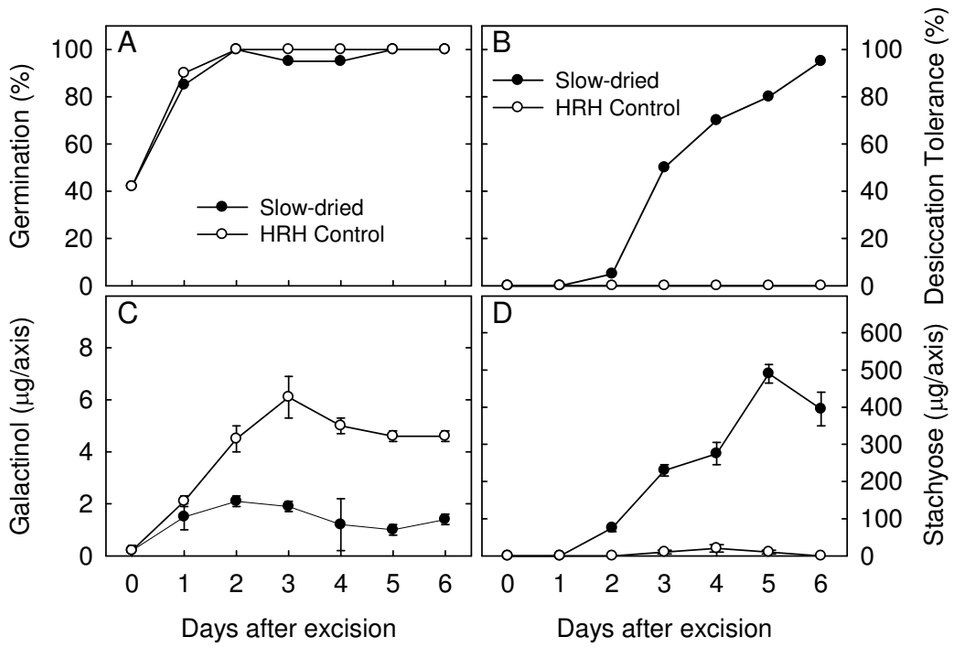

Fig. 16. Stachyose accumulation correlates with seed desiccation tolerance. A) Germination percentage for excised developing soybean seeds undergoing slow drying (filled circles) or high relative humidity (HRH; open circles) for 0 to 6 days after excision. Samples of 20-25 seeds were removed on successive days of each treatment and placed on moist germination paper. Percentage of germination (i.e., radicle emergence) was determined after 7 days on moist germination paper. B) Development of desiccation tolerance during slow drying (filled circles) or high RH control (open circles) treatments. Samples of 20-25 seeds were desiccated rapidly at 13% RH and then tested for germination. C) Content of galactinol in axes of excised developing soybean seeds undergoing slow drying (filled circles) or high RH control (open circles) for 0 to 6 days after excision. D) Content of stachyose in axes of excised developing soybean seeds undergoing slow drying (filled circles) or high RH control (open circles) for 0 to 6 days after excision. For C and D, values are mean ± SE of the mean of five samples of five axes each. Note the different scales on the y axis (adapted from Blackman et al., 1992; this material is copyrighted by the American Society of Plant Biologists and is reprinted with permission).

al., 1994) and contribute to phosphorus pollution through manure from animals fed phytic acid in seed and grain concentrates (Sebastian et al., 2000; Hitz et al., 2002). Seeds with reduced phytic acid can germinate in laboratory studies under minimal environmental stress. Therefore, there is considerable interest in lowering the phytic acid in soybean seed products commonly used in animal feeds. Soybean seeds expressing the mutant *mips* phenotype have reduced phytic acid (Sebastian et al., 2000; Hitz et al., 2002) but also have reduced field emergence compared to seeds expressing the normal *Mips* phenotype that accumulate normal amounts of phytic acid (Meis et al., 2003). Another approach using mutants homozygous for *lpa1* and *lpa2* (low phytic acid genes 1 and 2) also produce a low phytic acid phenotype, but these soybean seeds have reduced field emergence compared to seeds expressing the normal phytic acid phenotype (Oltmans et al., 2005). Additional research is needed to obtain low phytic acid phenotypes that result in field emergence

comparable to normal phytic acid phenotypes. Soluble carbohydrates metabolite profiling of low phytic acid (*lpa*) mutant soybean seeds detected reduced *myo*-inositol, galactinol, raffinose, stachyose, galactopinitol A, galactopinitol B, and fagopyritol B1 compared to the wild type (Frank et al., 2009). These results are similar to those observed for LRSP1 and LRSP2 seeds expressing the mutant *mips* phenotype (Tables 1 & 2).

9. Conclusions

Using soybean stem-leaf-pod explants, we fed free cyclitols to the cut stems of soybean explants and followed the changes in soluble carbohydrates downloaded from explant seed coats and also in mature dry seeds from explants. The results demonstrate that increasing the supply of D-*chiro*-inositol in maternal tissues can result in increased accumulation of fagopyritols in seeds expressing the mutant *stc1* phenotype with low RFO, in seeds expressing the mutant *mips* phenotype with reduced raffinose, stachyose and phytin, and in seeds expressing the normal *Stc1* and *Mips* phenotype with normal levels of raffinose, stachyose and phytin. Increasing *myo*-inositol may increase accumulation of phytic acid and/or RFO in seeds. Therefore, it is proposed that increasing the conversion of *myo*-inositol to D-*chiro*-inositol in soybean leaves and subsequent transport of D-*chiro*-inositol to the seeds for accumulation as fagopyritol B1 in maturing seeds may improve the field performance of mature soybean seeds expressing the mutant *mips* phenotype (Kosina et al., 2010).

10. Acknowledgment

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11. References

Adams, C.A., Rinne, R.W. & Fjerstad, M.C. (1980). Starch deposition and carbohydrase activities in developing and germinating soya bean seeds. *Annals of Botany* 45, 5, (May 1980) 577-582, ISSN 0305-7364

Adams, C.A., Fjerstad, M.C. & Rinne, R.W. (1983). Characteristics of soybean seed maturation: Necessity for slow dehydration. *Crop Science* 23, 2, (March-April 1983) 265-267, ISSN 0011-183x

Amuti, K.S. & Pollard, C.J. (1977). Soluble carbohydrates of dry and developing seeds. *Phytochemistry* 16, 5, (May 1977) 529-532, ISSN 0031-9422

Avigad, G. & Dey, P.M. (1997). Carbohydrate metabolism: storage carbohydrates, In: *Plant Biochemistry,* Dey, P.M. & Harborne, J.B. (Ed.), 143-204, Academic Press, ISBN 0122146743, San Diego, CA USA

Blackman, S.A., Obendorf, R.L. & Leopold, A.C. (1992). Maturation proteins and sugars in desiccation tolerance of developing soybean seeds. *Plant Physiology* 100, 1, (September 1992) 225-230, ISSN 0032-0889

Buitink, J., Thomas, M., Gissot, L. & Leprince, O. (2004). Starvation, osmotic stress and desiccation tolerance lead to expression of different genes of the regulatory beta and gamma subunits of the SnRK1 complex in germinating seeds of *Medicago truncatula*. *Plant Cell and Environment* 27, 1, (January 2004) 55–67, ISSN 0140-7791

Caffrey, M., Fonseca, V. & Leopold, A.C. (1988). Lipid-sugar interactions: Relevance to anhydrous biology. *Plant Physiology* 86, 3, (March 1988) 754-758, ISSN 0032-0889

Chappell, A.S., Scaboo, A.M., Wu, X., Nguyen, H., Pantalone, V.R. & Bilyeu, K.D. (2006). Characterization of the *MIPS* gene family in *Glycine max*. *Plant Breeding* 125, 5, (October 2006) 493–500, ISSN 0179-9541

Chibnall, A.C. & Westall, R.G. (1932). Estimation of glutamine in the presence of asparagine. *Biochemical Journal* 26, 1, (January 1932) 122-132, ISSN 0264-6021

Chiera, J.M., Streeter, J.G. & Finer, J.J. (2006). Ononitol and pinitol production in transgenic soybean containing the inositol methyl transferase gene from *Mesembryanthemum crystallinum*. *Plant Science* 171, 6, (December 2006) 647-654, ISSN 0168-9452

Chiera, J.M. & Grabau, E.A. (2007). Localization of *myo*-inositol phosphate synthase (GmMIPS-1) during the early stages of soybean seed development. *Journal of Experimental Botany* 58, 8, (August 2007) 2261-2268, ISSN 0022-0957

Crookston, R.K. & Hill, D.S. (1978). A visual indicator of the physiological maturity of soybean seed. *Crop Science* 18, 5, (September-October 1978) 867-870, ISSN 0011-183x

Dey, P.M. (1990). Oligosaccharides, In: *Methods in Plant Biochemistry Volume 2 Carbohydrates,* Dey, P.M. (Ed.), 189-218, Academic Press, ISBN 0124610129, New York, NY USA

Dierking, E.C. & Bilyeu, K.D. (2008). Association of a soybean raffinose synthase gene with low raffinose and stachyose seed phenotype. *Plant Genome* 1, 2, (November 2008) 135-145, ISSN 1940-3372

Dierking, E.C. & Bilyeu, K.D. (2009). Raffinose and stachyose metabolism are not required for efficient soybean seed germination. *Journal of Plant Physiology* 166, 12, (August 2009) 1329-1335, ISSN 0176-1617

Dittrich, P. & Brandl, A. (1987). Revision of the pathway of D-pinitol formation in Leguminosae. *Phytochemistry* 26, 7, (July 1987) 1925-1926, ISSN 0031-9422

Dornbos, D.L. & McDonald, M.B., Jr. (1986). Mass and composition of developing soybean seeds at five reproductive growth stages. *Crop Science* 26, 3, (May-June 1986) 624-630, ISSN 0011-183x

Egli, D.B. (1990). Seed water relations and the regulation of the duration of seed growth in soybean. *Journal of Experimental Botany* 41, 223, (February 1990) 243-248, ISSN 0022-0957

Egli, D.B. & TeKrony, D.M. (1993). Germination and water relations of immature soybean seed. *Seed Science and Technology* 21, 1, (January 1993) 139-148, ISSN 0251-0952

Ellis, E.C. & Spanswick, R.M. (1987). Sugar efflux from attached seed coats of *Glycine max* (L.) Merr. *Journal of Experimental Botany* 38, 194, (September 1987) 1470-1483, ISSN 0022-0957

Ford, C.W. (1985). Identification of inositols and their mono-*O*-methyl ethers by gas-liquid chromatography. *Journal of Chromatography* 333, 1, (October 1985) 167-170, ISSN 0021-9673

Frank, T., Norenberg, S. & Engel, K.H. (2009). Metabolite profiling of two novel low phytic acid (lpa) soybean mutants. *Journal of Agricultural and Food Chemistry* 57, 14, (July 2009) 6408-6416, ISSN 0021-8561

Ganter, J., Correa, J., Reicher, F., Heyraud, A. & Rinaudo, M. (1991). Low molecular weight carbohydrates from *Mimosa scabrella* seeds. *Plant Physiology and Biochemistry* 29, 2, (March-April 1991) 139-146, ISSN 0981-9428

Gitzelmann, R. & Auricchio, S. (1965) The handling of soya alpha-galactosides by a normal and a galactosemic child. *Pediatrics* 36, 2, (February 1965) 231-235, ISSN 0031-4005

Gomes, C.I., Obendorf, R.L. & Horbowicz, M. (2005). *myo*-Inositol, D-*chiro*-inositol, and D-pinitol synthesis, transport, and galactoside formation in soybean explants. *Crop Science* 45, 4, (July-August 2005) 1312-1319, ISSN 0011-183x

Górecki, R.J., Fordonski, G., Halmajan, H., Horbowicz, M., Jones, R.G. & Lahuta, L.B. (2001). Seed Physiology and Biochemistry, In: *Carbohydrates in Grain Legume Seeds: Improving Nutritional Quality and Agronomic Characteristics,* Hedley, C.L. (Ed.), 117-143, CAB International, ISBN 0851994679, Wallingford, Oxon, UK

Heaney, R.P., Weaver, C.M. & Fitzsimmons, M.L. (1991). Soybean phytate content - effect on calcium absorption. *American Journal of Clinical Nutrition* 53, 3, (March 1991) 745-747, ISSN 0002-9165

Hegeman, C.E., Good, L.L. & Grabau, E.A. (2001). Expression of D-*myo*-inositol-3-phosphate synthase in soybean. Implications for phytic acid biosynthesis. *Plant Physiology* 125, 4, (April 2001) 1941-1948, ISSN 0032-0889

Hitz, W.D., Carlson, T.J., Kerr, P.S. & Sebastian, S.A. (2002). Biochemical and molecular characterization of a mutation that confers a decreased raffinosaccharide and phytic acid phenotype on soybean seeds. *Plant Physiology* 128, 2, (February 2002) 650-660, ISSN 0032-0889

Hoch, G., Peterbauer, T. & Richter, A. (1999). Purification and characterization of stachyose synthase from lentil (*Lens culinaris*) seeds: Galactopinitol and stachyose synthesis. *Archives of Biochemistry and Biophysics* 366, 1, (June 1999) 75-81, ISSN 0003-9861

Horbowicz, M. & Obendorf, R.L. (1994). Seed desiccation tolerance and storability: Dependence on flatulence-producing oligosaccharides and cyclitols -- review and survey. *Seed Science Research* 4, 4, (December 1994) 385-405, ISSN 0960-2585

Horbowicz, M., Brenac, P. & Obendorf, R.L. (1998). Fagopyritol B1, *O*-α-D-galactopyranosyl-(1-2)-D-*chiro*-inositol, a galactosyl cyclitol in maturing buckwheat seeds associated with desiccation tolerance. *Planta* 205, 1, (May 1998) 1-11, ISSN 0032-0935

Hsu, S.H., Hadley, H.H. & Hymowitz, T. (1973). Changes in carbohydrate contents of germinating soybean seeds. *Crop Science* 13, 4, (July-August 1973) 407-410, ISSN 0011-183x

Htoo, J.K., Sauer, W.C., Zhang, Y., Cervantes, M., Liao, S.F., Araiza, B.A., Morales, A. & Torrentera, N. (2007). The effect of feeding low-phytate barley-soybean meal diets differing in protein content to growing pigs on the excretion of phosphorus and nitrogen. *Journal of Animal Science* 85, 11, (November 2007) 700-705, ISSN 0021-8812

Kadlec, P., Bjergegaard, C., Gulewicz, K., Horbowicz, M., Jones, A., Kintia, P., Kratchanov, C., Kratchanova, M., Lewandowicz, G., Soral-Smietana, M., Sorensen, H. & Urban, J. (2001). Carbohydrate chemistry, In: *Carbohydrates in Legume Seeds: Improving*

Nutritional Quality and Agronomic Characteristics, Hedley, C.L. (Ed.), 15-59, CAB International, ISBN 0851994679, Wallingford, Oxon, UK

Karner, U., Peterbauer, T. , Raboy, V., Jones, D.A. , Hedley, C.L. & Richter, A. (2004). *myo*-Inositol and sucrose concentrations affect the accumulation of raffinose family oligosaccharides in seeds. *Journal of Experimental Botany* 55, 405, (September 2004) 1981-1987, ISSN 0022-0957

Kosina, S.M., Castillo, A. , Schnebly, S.R. & Obendorf, R.L. (2009). Soybean seed coat cup unloading on plants with low-raffinose, low-stachyose seeds. *Seed Science Research* 19, 3, (September 2009) 145-153, ISSN 0960-2585

Kosina, S.M., Schnebly, S.R. & Obendorf, R.L. (2010). Free cyclitol unloading from seed coats on stem-leaf-pod explants of low-raffinose, low-stachyose, low-phytin soybean. Seed Science Research 20, 4, (December 2010) 223-236, ISSN 0960-2585

Koster, K.L. & Leopold, A.C. (1988). Sugars and desiccation tolerance in seeds. *Plant Physiology* 88, 3, (November 1988) 829-832, ISSN 0032-0889

Kuo, T.M., VanMiddlesworth, J.F. & Wolf, W.J. (1988). Content of raffinose oligosaccharides and sucrose in various plant seeds. *Journal of Agricultural and Food Chemistry* 36, 1, (January-February 1988) 32-36, ISSN 0021-8561

Loewus, F.A. & Murthy, P.P.N. (2000). *myo*-Inositol metabolism in plants. *Plant Science* 150, 1, (January 2000) 1-19, ISSN 0168-9452

Lowell, C.A. & Kuo, T.M. (1989). Oligosaccharide metabolism and accumulation in developing soybean seeds. *Crop Science* 29, 2, (March-April 1989) 459-465, ISSN 0011-183x

Lynch, S.R., Dassenko, S.A., Cook, J.D., Juillerat, M.A. & Hurrell, R.F. (1994). Inhibitory effect of a soybean-protein related moiety on iron-absorption in humans. *American Journal of Clinical Nutrition* 60, 4, (October 1994) 567-572, ISSN 0002-9165

Meis, S.J., Fehr, W.R. & Schnebly, S.R. (2003). Seed source effect on field emergence of soybean lines with reduced phytate and raffinose saccharides. Crop Science 43, 4, (July-August 2003) 1336-1339, ISSN 0011-183x

Neus, J.D., Fehr, W.R. & Schnebly, S.R. (2005). Agronomic and seed characteristics of soybean with reduced raffinose and stachyose. *Crop Science* 45, 2, (March-April 2005) 589-592, ISSN 0011-183x

Nicolas, P., Gertsch, I. & Parisod, C. (1984). Isolation and structure determination of an α-D-galactosyl-α-D-galactosyl-α-D-galactosyl-D-pinitol from the chick pea. *Carbohydrate Research* 131, 2, (August 1984) 331-334, ISSN 0008-6215

Nunes, A.C.S., Vianna, G.R., Cuneo, F., Amaya-Farfán, J., de Capdeville, G., Rech, E.L. & Aragão, F.J.L. (2006). RNAi-mediated silencing of the *myo*-inositol-1-phosphate synthase gene (*GmMIPS1*) in transgenic soybean inhibited seed development and reduced phytate content. *Planta* 224, 1, (June 2006) 125-132, ISSN 0032-0935

Obendorf, R.L., Ashworth, E.N. & Rytko, G.T. (1980). Influence of seed maturation on germinability in soybean. *Crop Science* 20, 4, (July-August 1980) 483-486, ISSN 0011-183x

Obendorf, R.L., Rytko, G.T. & Byrne, M.C. (1983). Soya bean seed growth and maturation by i*n vitro* pod culture. *Annals of Botany* 51, 2, (February 1983) 217-227, ISSN 0305-7364

Obendorf, R.L., Timpo, E.E., Byrne, M.C., Toai, T.V., Rytko, G.T., Hsu, F.C. & Anderson, B.G. (1984). Soya bean seed growth and maturation *in vitro* without pods. *Annals of Botany* 53, 6, (June 1984) 853-863, ISSN 0305-7364

Obendorf, R.L., Kock, J.L., Górecki, R.J., Amable, R.A. & Aveni, M.T. (1990). Methanol accumulation in maturing seeds. *Journal of Experimental Botany* 41, 225, (April 1990) 489-495, ISSN 0022-0957

Obendorf, R.L. (1997). Oligosaccharides and galactosyl cyclitols in seed desiccation tolerance (Review Update). *Seed Science Research* 7, 2, (June 1997) 63-74, ISSN 0960-2585

Obendorf, R.L., Dickerman, A.M., Pflum, T.M., Kacalanos, M.A. & Smith, M.E. (1998a). Drying rate alters soluble carbohydrates, desiccation tolerance, and seedling growth of soybean zygotic embryos during *in vitro* maturation. *Plant Science* 132, 1, (February 1998) 1-12, ISSN 0168-9452

Obendorf, R.L., Horbowicz, M., Dickerman, A.M., Brenac, P. & Smith, M.E. (1998b). Soluble oligosaccharides and galactosyl cyclitols in maturing soybean seeds *in planta* and *in vitro*. *Crop Science* 38, 1, (January-February 1998) 78-84, ISSN 0011-183x

Obendorf, R.L., Steadman, K.J., Fuller, D.J., Horbowicz, M. & Lewis, B.A. (2000). Molecular structure of fagopyritol A1 (*O*-α-D-galactopyranosyl-(1-3)-D-*chiro*-inositol) by NMR. *Carbohydrate Research* 328, 4, (October 2000) 623-627, ISSN 0008-6215

Obendorf, R.L., Odorcic, S., Ueda, T., Coseo, M.P. & Vassallo, E. (2004). Soybean galactinol synthase forms fagopyritol B1 but not galactopinitols: substrate feeding of isolated embryos and heterologous expression. *Seed Science Research* 14, 4, (December 2004) 321-333, ISSN 0960-2585

Obendorf, R.L., Sensenig, E.M., Wu, J., Ohashi, M., O'Sullivan, T.E., Kosina, S.M. & Schnebly, S.R. (2008a). Soluble carbohydrates in mature soybean seed after feeding D-*chiro*-inositol, *myo*-inositol, or D-pinitol to stem-leaf-pod explants of low-raffinose, low-stachyose lines. *Plant Science* 175, 5, (November 2008) 650-655, ISSN 0168-9452

Obendorf, R.L., Zimmerman, A.D., Ortiz, P.A., Taylor, A.G. & Schnebly, S.R. (2008b). Imbibitional chilling sensitivity and soluble carbohydrate composition of low raffinose, low stachyose soybean seed. *Crop Science* 48, 6, (November-December 2008) 2396-2403, ISSN 0011-183x

Obendorf, R.L., Zimmerman, A.D., Zhang, Q., Castillo, A., Kosina, S.M., Bryant, E.G., Sensenig, E.M., Wu, J. & Schnebly, S.R. (2009). Accumulation of soluble carbohydrates during seed development and maturation of low-raffinose, low-stachyose soybean. *Crop Science* 49, 1, (January-February 2009) 329-341, ISSN 0011-183x

Oltmans, S.E., Fehr, W.R., Welke, G.A., Raboy, V. & Peterson, K.L. (2005). Agronomic and seed traits of soybean lines with low-phytate phosphorus. *Crop Science* 45, 2, (March-April 2005) 593-598, ISSN 0011-183x

Peterbauer, T. & Richter, A. (2001). Biochemistry and physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds. *Seed Science Research* 11, 3, (Sepember 2001) 185-198, ISSN 0960-2585

Polanowski, A.J. & Obendorf, R.L. (1991). Soybean isocitrate lyase: purification, properties, immunoassay, and N-terminal sequence. *Plant Physiology and Biochemistry* 29, 2, (March-April 1991) 119-129, ISSN 0981-9428

Price, K.R., Lewis, J., Wyatt, G.M. & Fenwick, G.R. (1988). Flatulence -- Causes, relation to diet and remedies. *Die Nahrung* 32, 6, (June 1988) 609-623, ISSN 0027-769X

Quemener, B. & Brillouet, J.M. (1983) Ciceritol, a pinitol digalactoside from seeds of chickpea, lentil and white lupin. *Phytochemistry* 22, 8, (August 1983) 1745-1751, ISSN 0031-9422

Rainbird, R.M., Thorne, J.H., & Hardy, R.W.F. (1984). Role of amides, amino acids, and ureides in the nutrition of developing soybean seeds. *Plant Physiology* 74, 2, (February 1984) 329-334, ISSN 0032-0889

Raboy, V. (2009). Approaches and challenges to engineering seed phytate and total phosphorus. *Plant Science* 177, 4, (October 2009) 281-296, ISSN 0168-9452.

Rosnoblet, C., Aubry, C., Leprince, O., Vu, B.L., Rogniaux, H. & Buitink, J. (2007). The regulatory gamma subunit SNF4b of the sucrose non-fermenting-related kinase complex is involved in longevity and stachyose accumulation during maturation of *Medicago truncatula* seeds. *Plant Journal* 51, 1, (July 2007) 47-59, ISSN 0960-7412

Rubel, A., Rinne, R.W. & Canvin, O.T. (1972). Protein, oil, and fatty acid in developing soybean seeds. *Crop Science* 12, 6, (November-December 1972) 739-741, ISSN 0011-183x

Ruttloff, H., Täufel, A., Krause, W., Haenel, H. & Täufel, K. (1967). The intestinal enzymatic decompositon of galacto-oligosaccharides in the human and animal intestine, with particular regard to *Lactobacillus bifidus*. Part II. On the intestinal behaviour of lactulose. *Die Nahrung* 11, (1967) 39-46, ISSN 0027-769X

Saab, I.N. & Obendorf, R.L. (1989) Soybean seed water relations during in situ and in vitro growth and maturation. *Plant Physiology* 89, 2, (February 1989) 610-616, ISSN 0032-0889

Sasaki,K., Hicks,K.B. & Nagahashi,G. (1988). Separation of eight inositol isomers by liquid chromatography under pressure using a calcium-form, cation-Exchange column. *Carbohydrate Research* 183, 1, (November 1988) 1-9, ISSN 0008-6215

Schweizer, T.F., Horman, I. & Würsch, P. (1978). Low molecular weight carbohydrates from leguminous seeds; a new disaccharide: galactopinitol. *Journal of the Science of Food and Agriculture* 29, 2, (February 1978) 148-154, ISSN 0022-5142

Schweizer, T.F. & Horman, I. (1981). Purification and structure determination of three α-D-galactopyranosylcyclitols from soya beans. *Carbohydrate Research* 95, 1, (September 1981) 61-71, ISSN 0008-6215

Sebastian, S.A., Kerr, P.S., Pearlstein, R.W. & Hitz, W.D. (2000). Soybean germplasm with novel genes for improved digestibility, in *Soy in Animal Nutrition,* Drackley, J.K. (Ed.), 56-73, Federation of Animal Science Societies, ISBN 18884706010, Savoy, Illinois, USA

Sharpley, A.N., Daniel, T., Sims, T., Lemunyon, J., Stevens, R. & Perry, R. (2003). *Agricultural Phosphorus and Eutrophication*, 2nd Edition. United States Department of Agriculture, Agricultural Research Service, ARS 149

(http://ddr.nal.usda.gov/dspace/bitstream/10113/26693/1/CAT30907360.pdf)

Slawinska, J. & Obendorf, R.L. (1991). Soybean somatic embryo maturation: composition, respiration and water relations. *Seed Science Research* 1, 4, (December 1991) 251-262, ISSN 0960-2585

Steadman, K.J., Fuller, D.J. & Obendorf, R.L. (2001). Purification and molecular structure of two digalactosyl D-*chiro* -inositols and two trigalactosyl D-*chiro*-inositols from buckwheat seeds. *Carbohydrate Research* 331, 1, (March 2001) 19-25, ISSN 0008-6215

Streeter, J.G. (2001). Simple partial purification of D-pinitol from soybean leaves. *Crop Science* 41, 6, (November-December 2001) 1985-1987, ISSN 0011-183x

Streeter, J.G., Lohnes, D.G. & Fioritto, R.J. (2001). Patterns of pinitol accumulation in soybean plants and relationships to drought tolerance. *Plant Cell and Environment* 24, 4, (April 2001) 429-438, ISSN 0140-7791

Suarez, F.L., Springfield, J., Furne, J.K., Lohrmann, T.T., Kerr, P.S. & Levitt, M.D. (1999). Gas production in humans ingesting a soybean flour derived from beans naturally low in oligosaccharides. *American Journal of Clinical Nutrition* 69, 1, (January 1999) 135-139, ISSN 0002-9165

Szczecinski, P., Gryff-Keller, A., Horbowicz, M. & Obendorf, R.L. (1998) NMR investigation of the structure of fagopyritol B1 from buckwheat seeds. *Bulletin of the Polish Academy of Sciences, Chemistry* 46, 1, (January 1998) 9-13, ISSN 0239-7285

Szczecinski, P., Gryff-Keller, A., Horbowicz, M. & Lahuta, L.B. (2000). Galactosylpinitols isolated from vetch (*Vicia villosa* Roth.) seeds. *Journal of Agricultural and Food Chemistry* 48, 7, (July 2000) 2717-2720, ISSN 0021-8561

Taguchi, R., Yamazaki, J., Tsutsui, Y. & Ikezawa, H. (1997). Identification of *chiro*-inositol and its formation by isomerization of *myo*-inositol during hydrolysis of glycosylphosphatidylinositol-anchored proteins. *Archives of Biochemistry and Biophysics* 342, 1, (June 1997) 161-168, ISSN 0003-9861

TeKrony, D.M., Egli, D.B., Balles, J., Pfeiffer, T. & Fellows, R.J. (1979). Physiological maturity in soybean. *Agronomy Journal* 71, 5, (September-October 1979) 771-775, ISSN 0002-1962

Thorne, J.H. & Rainbird, R.M. (1983). An *in vivo* technique for the study of phloem unloading in seed coats of developing soybean seeds. *Plant Physiology* 72, 1, (January 1983) 268-271, ISSN 0032-0889

Traitler, H., Del Vedovo, S. & Schweizer, T.F. (1984). Gas chromatographic separation of sugars by on-column injection on glass capillary column. *Journal of High Resolution Chromatography and Chromatography Communications* 7, 314, (November 1984) 558-562, ISSN 0021-9673

Vernon, D.M. & Bohnert, H.J. (1992). A novel methyl transferase induced by osmotic stress in the facultative halophyte *Mesembryanthemum crystallinum*. *European Molecular Biology Organization Journal* 11, 6, (June 1992) 2077-2085, ISSN 0261-4189

Vernon, D.M., Tarczynski, M.C., Jensen, R.G. & Bohnert, H.J. (1993). Cyclitol production in transgenic tobacco. *Plant Journal* 4, 1, (July 1993) 199-205, ISSN 0960-7412

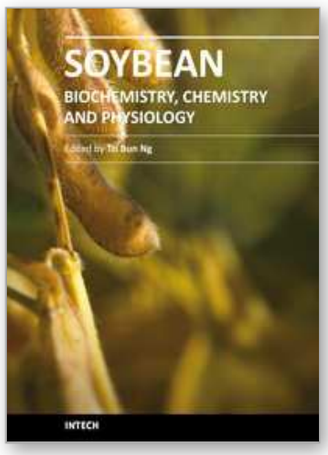
VerNooy, C.D., Thorne, J.H., Lin, W. & Rainbird, R.M. (1986). Cessation of assimilate uptake in maturing soybean seeds. *Plant Physiology* 82, 1, (September 1986) 222-225, ISSN 0032-0889

Von Ohlen, F.W. (1931). A microchemical study of soybeans during germination. *American Journal of Botany* 18, 1, (January 1931) 30-49, ISSN 0002-9122

Wettlaufer, S.H. & Obendorf, R.L. (1991). Ureides and amides as nitrogen sources for soybean seed growth and maturation *in vitro*. *Crop Science* 31, 5, (September-October 1991) 1319-1323, ISSN 0011-183x

Wanek, W. & Richter, A. (1995). Purification and characterization of *myo*-inositol 6-*O*-methyltransferase from *Vigna umbellata* Ohwi et Ohashi. *Planta* 197, 3, (October 1995) 427-434, ISSN 0032-0935

Yazdi-Samadi, G., Rinne, R.W. & Seif, R.D. (1977). Components of developing soybean seeds: Oil, protein, sugars, starch, organic acids, and amino acids. *Agronomy Journal* 69, 3, (May-June 1977) 481-486, ISSN 0002-1962



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