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Soybean seed coat cup unloading on plants with low-raffinose, low-stachyose seeds

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Abstract

Sucrose, raffinose and stachyose accumulate in soybean [*Glycine max* L. (Merrill)] embryos during seed maturation. To determine the relationship of plant maternal composition on seed composition, soluble carbohydrates in three 1-cm^2 leaf punches at three plant growth stages (R2, R3, R6) and in seed coat cup exudates *in planta* were analysed at four 30-min intervals on soybean plants (R5) with low-raffinose, low-stachyose (LRS) seeds expressing the mutant *stc1* phenotype; low-raffinose, low-stachyose and low-phytin (LRSP1, LRSP2) seeds expressing the mutant *mips* phenotype; or normal raffinose, stachyose and phytin (CHECK) seeds expressing the *Stc1* and *Mips* phenotype. Leaf sucrose (23.6 μg cm^{-2}), *myo*-inositol (9.3 μg cm^{-2}), D-*chiro*-inositol (6.7 μg cm^{-2}), D-ononitol (0.76 μg cm^{-2}), D-pinitol (50.1 μg cm^{-2}) and total soluble carbohydrates (107.1 μg cm^{-2}) were not significantly different between phenotypes. D-*chiro*-Inositol, *myo*-inositol, D-pinitol and sucrose were unloaded from soybean seed coat cups *in planta* at decreasing rates over the four sequential periods of sampling. Unloading rates of sucrose and *myo*-inositol were highest for LRS, D-pinitol was highest for LRSP2, and D-*chiro*-inositol was not different between LRS, LRSP1, LRSP2 and CHECK. Free cyclitols were 60% of total soluble carbohydrates in leaves and 20% in seed coat cup exudates. Except for sucrose and D-pinitol, seed phenotype had little influence on the composition of compounds unloaded from seed coats to maturing embryos of low-raffinose, low-stachyose seeds. Maternally supplied cyclitols may contribute, in part, to changes in the composition of cyclitol galactosides stored in mature seeds.

Keywords: **D-*chiro*-inositol, *Glycine max* (L.) Merrill, *myo*-inositol, D-pinitol, seed coat cup unloading, sucrose**

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Introduction

Soybean [*Glycine max* (L.) Merrill] leaves synthesize free cyclitols (D-*chiro*-inositol, *myo*-inositol, D-ononitol, D-pinitol) in addition to sucrose (Streeter, 2001; Streeter *et al.*, 2001; Gomes *et al.*, 2005). Sucrose, D-*chiro*-inositol, *myo*-inositol and D-pinitol are transported to the seeds, and are unloaded from the seed coat to the developing embryo where they accumulate as their respective α-galactosides during seed maturation and desiccation (Obendorf *et al.*, 1998, 2009; Gomes *et al.*, 2005) (Fig. 1). D-Ononitol, galactinol, galactopinitols, fagopyritol B1, raffinose and stachyose were not detected in soybean seed coat cup exudates (Gomes *et al.*, 2005). *myo*-Inositol phosphate synthase (MIPS) converts glucose-6-phosphate to 1D-*myo*-inositol-3-phosphate, a key reaction for the synthesis of *myo*-inositol (Fig. 1) and downstream products galactinol, raffinose, stachyose and phytic acid (Loewus and Murthy, 2000; Peterbauer and Richter, 2001; Hitz *et al.*, 2002; Raboy, 2003). Of the four *MIPS* genes in soybean plants, *MIPS1* is highly expressed in immature seeds compared to the low expression of *MIPS2*, *MIPS3* and *MIPS4* (Hegeman *et al.*, 2001; Hitz *et al.*, 2002; Chappell *et al.*, 2006; Nunes *et al.*, 2006; Chiera and Grabau, 2007). By contrast, *MIPS4* is highly expressed in leaves. In leaves, *myo*-inositol is converted via the intermediate D-ononitol to D-pinitol (Dittrich and Brandl, 1987) (Fig. 1). D-Pinitol accumulates as an end product in leaves, especially during drought stress (Streeter, 2001; Streeter *et al.*, 2001). There is no evidence for the conversion of D-pinitol to D-*chiro*-inositol (Dittrich and Brandl, 1987). *myo*-Inositol is converted to D-*chiro*-inositol in animals, insects, algae and bacteria (reviewed in Hipps *et al.*, 1973; Pak *et al.*, 1992; Obendorf, 1997; Sun *et al.*, 2002; Yoshida *et al.*, 2006). Evidence from soybean and buckwheat (*Fagopyrum esculentum* Moench) explant feeding experiments supports the conversion of *myo*-inositol to D-*chiro*-inositol (Gomes *et al.*, 2005; Ma *et al.*, 2005) (Fig. 1). The conversion of D-ononitol to D-pinitol and the conversion of *myo*-inositol to D-*chiro*-inositol in plants are probably by unknown oxidoreductases (Fig. 1) (Obendorf, 1997).

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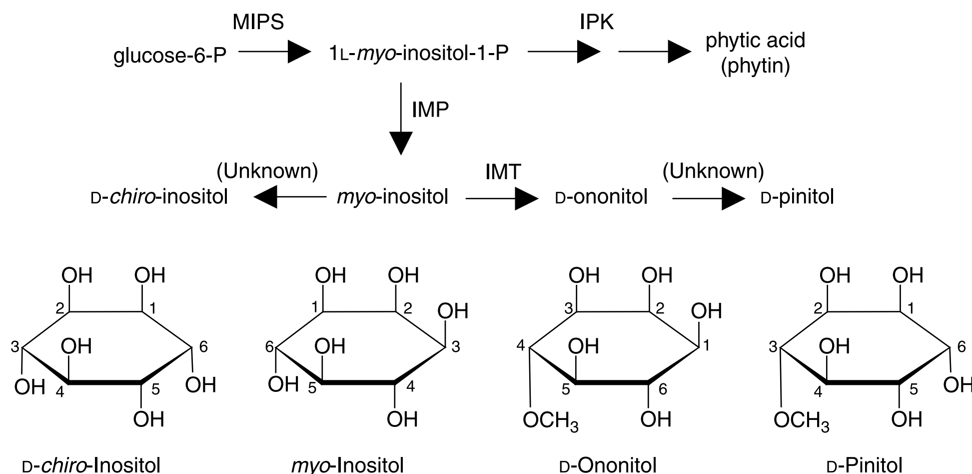

Figure 1. Proposed pathway for synthesis of cyclitols in leaves and phytic acid in seeds. Parentheses ( ) by an arrow indicate that an enzyme catalysing the reaction has not been identified. IMP, *myo*-Inositol-phosphate monophosphatase (EC 3.1.3.25); IMT, *myo*-inositol 4-*O*-methyltransferase (EC 2.1.1.129); IPK, inositol polyphosphate kinases; MIPS, *myo*-inositol-phosphate synthase (EC 5.5.1.4); D-ononitol, 1D-4-*O*-methyl-*myo*-inositol; D-pinitol, 1D-3-*O*-methyl-*chiro*-inositol; (unknown), unknown oxidoreductase.

Mature soybean seeds contain soluble carbohydrates (15% of dry mass), mostly sucrose, raffinose and stachyose plus small amounts of galactopinitols and fagopyritols (Obendorf *et al.*, 1998). Raffinose, stachyose and phytin are largely undigested in non-ruminant animals and contribute to reduced feed efficiency and phosphorus pollution in manure (Price *et al.*, 1988; Heaney *et al.*, 1991; Zhou *et al.*, 1992; Parsons *et al.*, 2000; Sebastian *et al.*, 2000). Therefore, soybean products with low raffinose, stachyose and phytin are desired for feeding chickens and pigs (Sebastian *et al.*, 2000). Consumption of low-raffinose, low-stachyose soybean reduces flatulence in humans (Suarez *et al.*, 1999). Seeds expressing the mutant *mips* (wild-type *Mips* sequence designation Gm mI 1-PS-1A, AY038802) phenotype have low *myo*-inositol, galactinol, raffinose, stachyose and phytin (50% of normal) (Sebastian *et al.*, 2000; Hitz *et al.*, 2002) but are sensitive to imbibitional chilling (Obendorf *et al.*, 2008b) and have lower field emergence (Meis *et al.*, 2003). Seeds expressing the mutant *stc1* phenotype have low raffinose and stachyose but higher galactinol (Sebastian *et al.*, 2000; Hitz *et al.*, 2002), accumulate ninefold more galactosyl cyclitols than seeds expressing the mutant *mips* phenotype (Obendorf *et al.*, 2008b), tolerate imbibitional chilling (Obendorf *et al.*, 2008b), and have field emergence comparable to seeds expressing the *Stc1* and *Mips* phenotype with normal raffinose, stachyose and phytin (Neus *et al.*, 2005). The accumulation of cyclitol galactosides, especially fagopyritols, in embryos of maturing soybean seeds may substitute for low raffinose and stachyose in conveying tolerance to imbibitional chilling (Obendorf *et al.*, 2008b).

Sucrose, D-*chiro*-inositol, *myo*-inositol, D-pinitol are unloaded from the seed coat (maternal tissues) to the developing embryo of seeds expressing the *Stc1* and *Mips* phenotype with normal raffinose, stachyose and phytin (Gomes *et al.*, 2005), but the profiles of unloaded compounds from seed coats to embryos of seeds expressing the mutant *stc1* and *mips* phenotypes is unknown. The hypothesis is that maternal synthesis of *myo*-inositol may be normal and independent of the low *myo*-inositol synthesis in soybean seeds expressing the mutant *mips* phenotype (Hitz *et al.*, 2002). It is also predicted that all lines have similar synthesis of D-pinitol and D-*chiro*-inositol in leaves and that all lines have similar cyclitol unloading patterns independent of seed phenotype. The objective of this investigation was to characterize the *in planta* profiles of sucrose, D-*chiro*-inositol, *myo*-inositol and D-pinitol in leaf blade tissues and unloaded from maternal tissues of the seed coat to embryos of seeds expressing mutant *stc1* phenotype (low raffinose and stachyose) and mutant *mips* phenotype (low raffinose, stachyose and phytin) in comparison to normal *Stc1* and *Mips* phenotype (normal raffinose, stachyose and phytin).

Methods

Plant materials

Seeds for each of four proprietary soybean [*Glycine max* (L.) Merrill] lines with low raffinose and stachyose (LRS) seeds expressing the mutant *stc1* phenotype; low raffinose, stachyose and phytin (LRSP1, LRSP2) seeds expressing the mutant *mips* phenotype; and normal raffinose, stachyose and phytin (CHECK) seeds expressing the *Stc1* and *Mips* phenotype were provided by Steve Schnebly, Pioneer Hi-Bred, in

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November 2003. 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 the breeding lines utilized in this study were described by Sebastian *et al.* (2000), Hitz *et al.* (2002) and Meis *et al.* (2003). To accommodate a series of different experiments, five replicates of each were planted weekly and mature dry seeds were harvested as needed for maintenance of the lines (soybean is naturally cleistogamous). Plants from the first or second generation of greenhouse-grown seeds were grown in a climate-controlled greenhouse at 21°C for 10-hour nights and at 27°C for 14-hour days supplemented with 640 $\mu mol\, m^{-2} s^{-1}$ incandescent light from Sylvania metal halide (1000 watt BU) lamps.

Leaf soluble carbohydrates

Three 1-cm^2 leaf punches, one from each leaflet, were taken from leaves (avoiding the larger veins) at the fifth node of 14 replicate soybean plants at growth stages R2 (full bloom) or R3 (beginning pod) and nine replicate plants at growth stage R6 (full seeds) (Fehr and Caviness, 1977) for each of the four lines (LRS, LRSP1, LRSP2, CHECK). Other plant growth stages were not sampled. Leaf punches from one or two replicate plants of each line were harvested at weekly intervals using plants from different seeding dates. Immediately after sampling, punches were placed in a vial, immersed in liquid nitrogen, and stored at −80°C. The frozen leaf punches were ground to a fine powder using a mortar and pestle cooled with liquid nitrogen. The frozen powder was extracted in a ground glass homogenizer with 900 µl of ethanol:water (1:1, v/v) containing 100 µg of phenyl α-D-glucoside as internal standard. Samples were centrifuged for 20 min at 14,000 × *g* at 4°C, and supernatants were passed through a 10,000 molecular weight cutoff filter (NANOSEP 10K Omega, Pall Corp., East Hills, New York, USA) at 14,000 × *g*. The filtrate (200 µl) was transferred to a silylanized 400-µl flat-bottomed insert in a silylation vial and dried under a stream of nitrogen gas. Dried samples were placed above P_2O_5 in desiccators overnight to remove traces of water. Dry residues were derivatized with 100 µl of trimethylsilylimidazole (TMSI):pyridine (1:1, v/v) for 45 min at 80°C and analysed by gas chromatography (Horbowicz and Obendorf, 1994) with minor changes (Gomes *et al.*, 2005).

In planta *seed coat cup exudate analysis*

Pods from eight replicate plants at growth stage R5 (early seed fill) (Fehr and Caviness, 1977) for each of four lines were selected for analysis. Seed coat cup unloading analysis was performed on the central seed (280–300 mg fresh weight) of one pod per plant using the surgical method of removing the distal half of the seed coat and the entire embryo from the intact seed coat cup, as described by Thorne and Rainbird (1983) and Gomes *et al.* (2005). Because buffer, salts and mannitol (Thorne and Rainbird, 1983) interfered with cyclitol analysis, unloaded compounds were collected in water. The seed coat cup was filled with 200 µl double-distilled water (ddH_2O), rinsed twice within 10 min, and then refilled. Four 200-µl samples were collected at 30-min intervals for 2 h (cups refilled after each sampling). An equal volume of ethanol was added to each collected sample plus 50 µg of phenyl α-D-glucoside as internal standard. Samples were dried, and soluble carbohydrates were analysed as described above.

Statistical analyses

Statistical analysis (ANOVA) was performed after a square root transformation of the responses, to correct for non-constant residual variance. Significant differences ($P < 0.05$) after a Tukey correction for multiple comparisons were determined between lines, plant growth stages and line-by-growth stage interactions, using JMP Statistical Discovery Software, Release 7.0 (SAS Institute Inc., Cary, North Carolina, USA). When the line-by-plant-growth-stage interaction was not significantly different for responses in leaf punches, data were pooled across three growth stages to compare lines and pooled across four lines to compare growth stages. When the line-by-sampling-period interaction was not significantly different for unloading responses, data were pooled across four sampling periods to compare lines and pooled across four lines to compare sampling periods. Pooled responses are reported as mean ± standard error of the mean (vertical bars). Significant differences are noted by different letters.

Results

Leaf soluble carbohydrates

Raffinose family oligosaccharides (RFO; raffinose, stachyose, verbascose) and galactosyl cyclitols (galactinol, galactopinitols, fagopyritols) were not detected in leaf extracts. Sucrose (23.6 ± 0.8, as $\mu g\, cm^{-2}$ ± SE when pooled across all treatments), *myo*-inositol (9.3 ± 0.5 $\mu g\, cm^{-2}$), D-*chiro*-inositol (6.7 ± 0.3 $\mu g\, cm^{-2}$), D-ononitol (0.76 ± 0.09 $\mu g\, cm^{-2}$), D-pinitol (50.1 ± 2.4 $\mu g\, cm^{-2}$), and total soluble carbohydrates (107.1 ± 3.6 $\mu g\, cm^{-2}$) were present in leaf extracts. Free cyclitols (*myo*-inositol, D-*chiro*-inositol, D-ononitol, D-pinitol) were 60% of the total soluble

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carbohydrates extracted from leaf punches. Because line-by-plant-growth-stage interactions were not significantly different for each soluble carbohydrate in leaf extracts, responses are reported as a function of line (Fig. 2) and plant growth stage (Fig. 3).

myo-Inositol, D-*chiro*-inositol, D-ononitol, D-pinitol, sucrose and total soluble carbohydrates in leaf extracts were not significantly different among lines (Fig. 2A–F). *myo*-Inositol and D-*chiro*-inositol at plant growth stage R6 were significantly less than at plant growth stages R2 and R3 (Fig. 3A, D). D-Ononitol concentrations were low and decreased significantly with plant growth stage (R2 > R3 > R6) (Fig. 3B). D-Pinitol concentrations in leaf extracts were high and increased slightly between plant growth stages R2 and R6 (Fig. 3E). D-Pinitol averaged 42% of total soluble carbohydrates, increasing from 39% at R2 to 49% at R6. Since D-ononitol is an intermediate in the synthesis of D-pinitol from *myo*-inositol (Fig. 1) (Dittrich and Brandl, 1987), the observed decrease in D-ononitol (Fig. 3B) in leaf punch extracts was consistent with the observed decrease in the concentration of the substrate *myo*-inositol (Fig. 3A).

Sucrose ($23.6 \pm 0.8\,\mu g\,cm^{-2}$), glucose ($8.5 \pm 0.5\,\mu g\,cm^{-2}$), fructose ($4.3 \pm 0.4\,\mu g\,cm^{-2}$), maltose ($4.5 \pm 0.3\,\mu g\,cm^{-2}$), total soluble carbohydrates ($107.1 \pm 3.6\,\mu g\,cm^{-2}$) and total cyclitols ($66.9 \pm 2.7\,\mu g\,cm^{-2}$) in leaf extracts were not

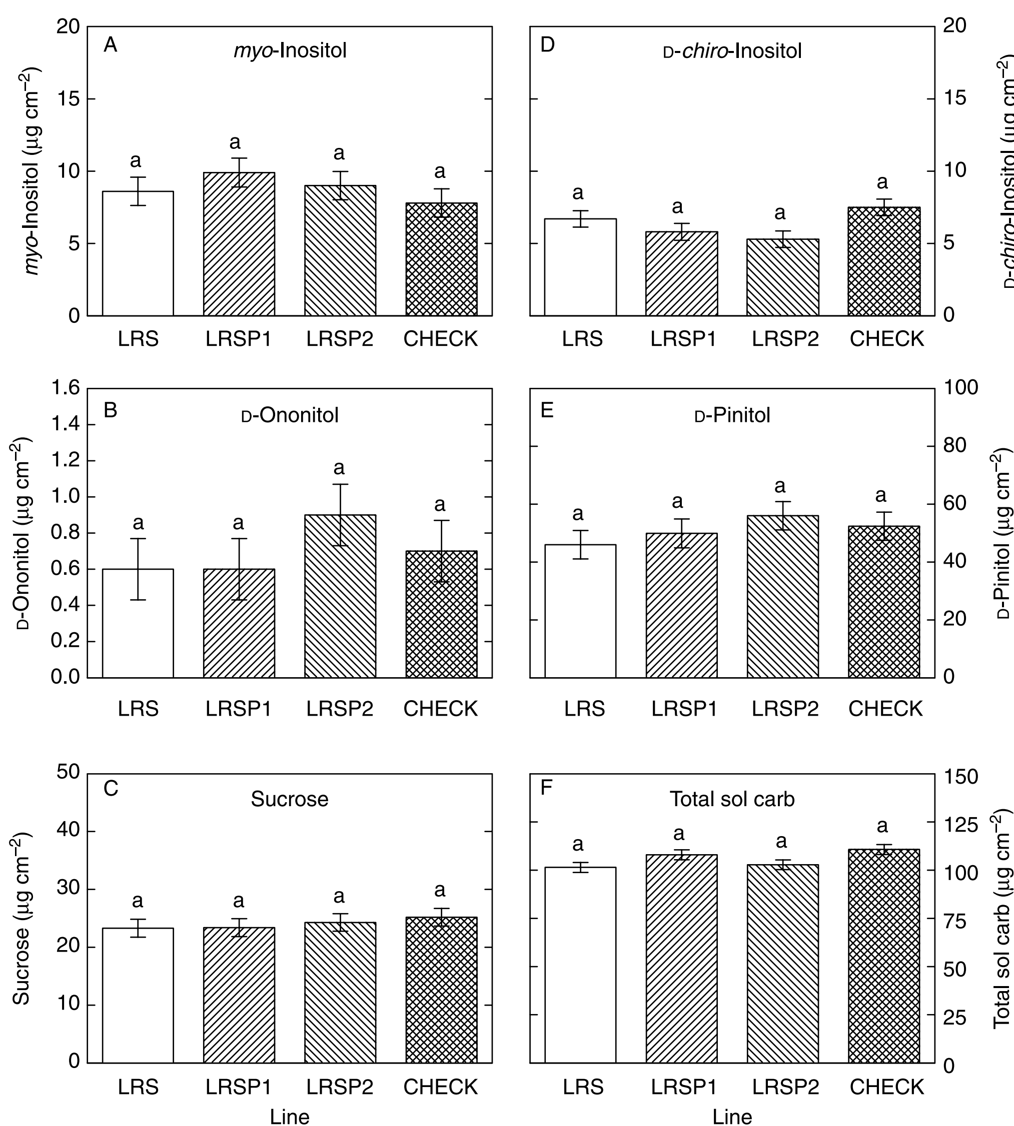

Figure 2. *In planta* leaf punch extract concentrations ($\mu g\,cm^{-2}$) for *myo*-inositol (A), D-ononitol (B), sucrose (C), D-*chiro*-inositol (D), and D-pinitol (E) and total soluble carbohydrates (F) for four soybean lines (LRS, low stachyose; LRSP1, LRSP2, low raffinose, stachyose and phytin; CHECK normal raffinose, stachyose and phytin). Since line-by-plant-growth-stage interactions were not significantly different, data were pooled across three plant growth stages for each line. Responses among lines were not significantly different ($P < 0.05$; $n = 37$) as noted by the same letter by each bar.

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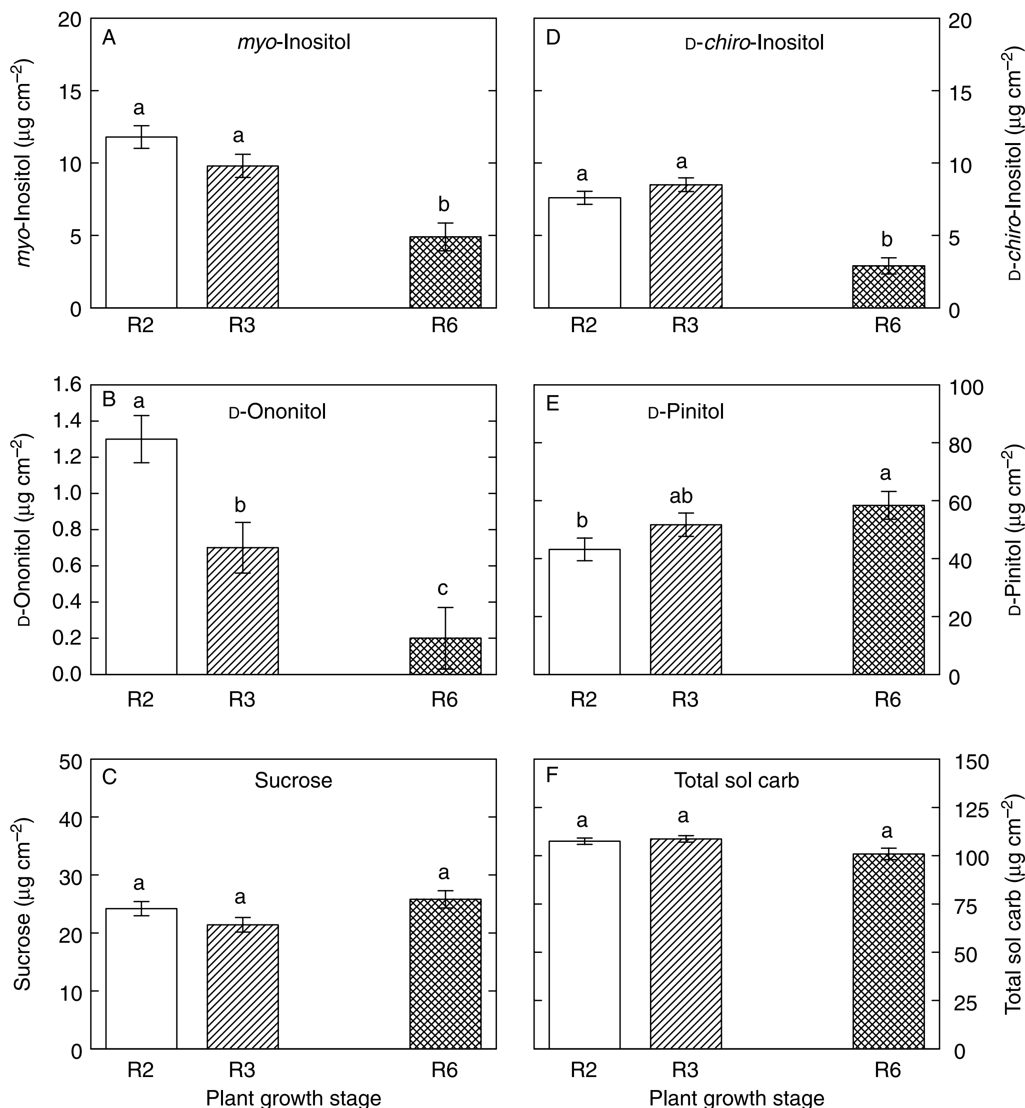

Figure 3. *In planta* leaf punch extract concentrations ($\mu g\,cm^{-1}$) for *myo*-inositol (A), D-ononitol (B), sucrose (C), D-*chiro*-inositol (D), D-pinitol (E), and total soluble carbohydrates (F) for three soybean plant growth stages (R2, full bloom; R3, beginning to pod; R6, full seed). Since line-by-plant-growth-stage interactions were not significantly different, data were pooled across four lines. Responses with different letters are significantly different ($P < 0.05$; $n = 14$ for R2 and R3, $n = 9$ for R6, for each of four lines) after a Tukey correction for comparison of multiple means.

significantly different among lines, plant growth stages, or line-by-growth-stage interactions (Fig. 3C, F; reducing sugars and total cyclitols not shown).

Seed coat cup unloading of endogenous cyclitols in planta

Mean unloading rates for sucrose, D-pinitol, *myo*-inositol and D-*chiro*-inositol by attached seed coat cups *in planta* were 147.7 ± 4.7, 32.9 ± 1.7, 3.6 ± 0.1 and 5.1 ± 0.2 $\mu g\,h^{-1}$, respectively, when pooled across all lines and sampling periods. D-Ononitol, galactinol, galactopinitols, fagopyritols, raffinose, stachyose and verbascose were not detected in seed coat cup exudates. In the absence of significant line-by-sampling-period interactions, responses were pooled across sampling periods for each line (LRS, LRSP1, LRSP2, CHECK) and pooled across lines for each sampling period (0–30, 30–60, 60–90, 90–120 min) (Fig. 4). Sucrose, D-pinitol, *myo*-inositol and D-*chiro*-inositol were unloaded from soybean seed coat cups *in planta* at decreasing rates over the four sequential periods of sampling (Fig. 4E–H). Sucrose and *myo*-inositol unloading rates were highest for LRS (Fig. 4A, C), D-pinitol unloading rate was highest for LRSP2 (Fig. 4B), and D-*chiro*-inositol unloading rate was not significantly different among lines (Fig. 4D). Cyclitols were about 20% of total soluble carbohydrates unloaded.

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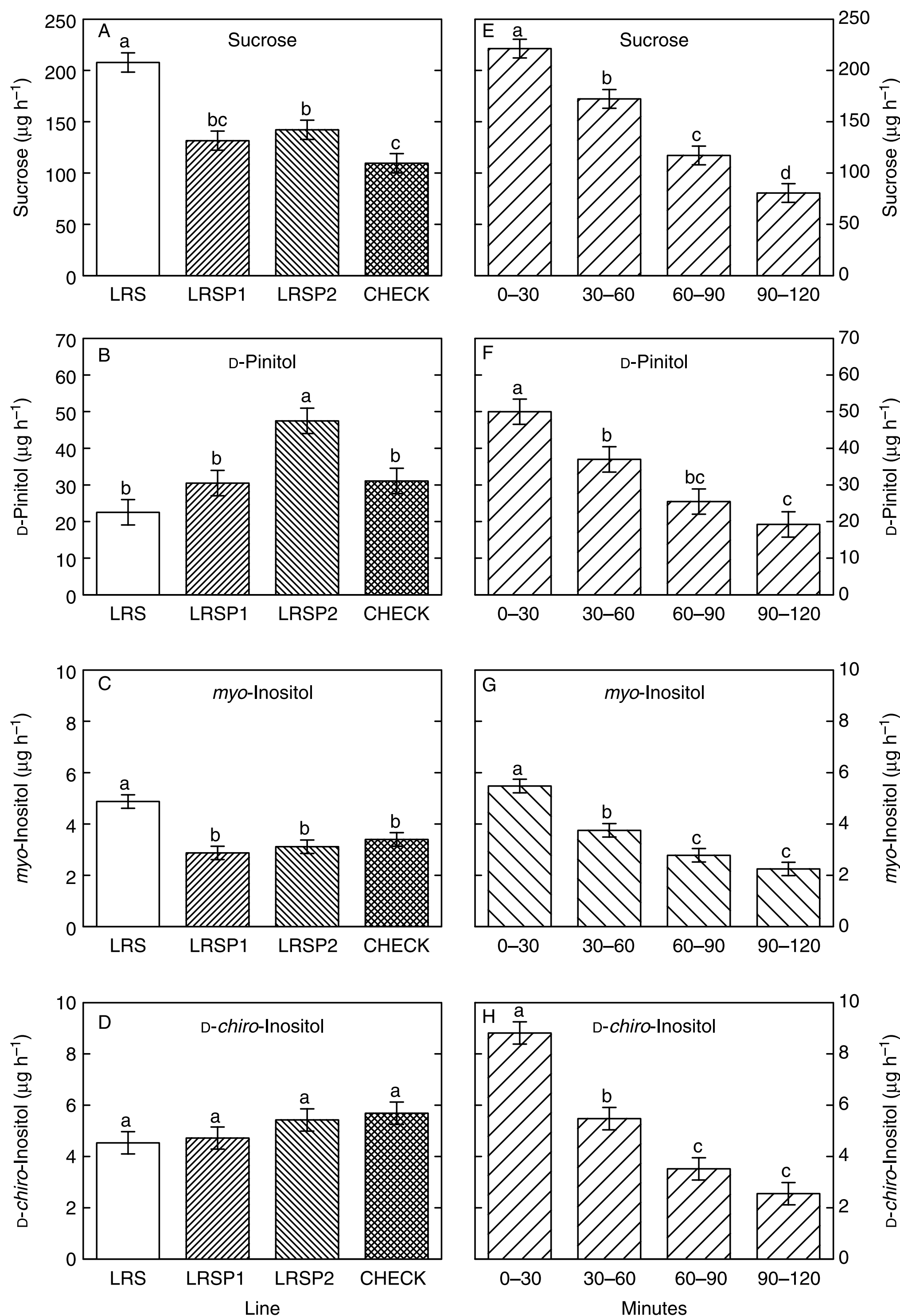

Figure 4. *In planta* seed coat cup unloading rates for sucrose (A, E), D-pinitol (B, F), *myo*-inositol (C, G), and D-*chiro*-inositol (D, H) for four soybean lines (A–D) and four sampling times (E–H). Responses with different letters are significantly different ($P < 0.05$; $n = 8$) after a Tukey correction for comparison of multiple means.

Discussion

Soybean seeds accumulate α-galactosides of sucrose (raffinose, stachyose, verbascose), *myo*-inositol (galactinol), D-*chiro*-inositol (fagopyritols) and D-pinitol (galactopinitols) during normal seed maturation and desiccation (Obendorf *et al.*, 1998; Gomes *et al.*, 2005). Sucrose, D-pinitol, *myo*-inositol and D-*chiro*-inositol, but not D-ononitol, are transported from maternal tissues to the developing embryo (Fig. 4)

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(Gomes *et al.*, 2005). *myo*-Inositol may also be synthesized in immature soybean embryos (Hitz *et al.*, 2002; Chiera *et al.*, 2006), but there is no evidence for the synthesis of D-*chiro*-inositol and D-pinitol in embryos (Obendorf *et al.*, 2004; Gomes *et al.*, 2005; Chiera *et al.*, 2006). Feeding free D-*chiro*-inositol and D-pinitol increased fagopyritols and galactopinitols, respectively, in soybean cotyledons (Obendorf *et al.*, 2004; Gomes *et al.*, 2005). Soybean somatic embryos transformed with a *myo*-inositol *O*-methyl transferase (IMT, Fig. 1) gene accumulated D-ononitol and D-pinitol during embryo maturation (Chiera *et al.*, 2006). D-Pinitol accumulation in leaves was not different between transformed and non-transformed plants (Chiera *et al.*, 2006). Soybean seeds expressing the mutant *stc1* phenotype have reduced raffinose synthase activity and low raffinose and stachyose (LRS) accumulation (Hitz *et al.*, 2002), but high galactinol synthase activity (Hitz *et al.*, 2002) and higher galactinol accumulation (Hitz *et al.*, 2002; Obendorf *et al.*, 2008b, 2009). Seeds expressing the mutant *mips* phenotype have low raffinose, stachyose and phytin (LRSP1, LRSP2) (Sebastian *et al.*, 2000; Hitz *et al.*, 2002) in addition to low concentrations of galactinol (Hitz *et al.*, 2002; Obendorf *et al.*, 2008b, 2009).

Soybean seed coat cups unload the amides glutamine and asparagine (70% of N) and amino acids in addition to sucrose (90% of C), but cyclitols were not determined (Rainbird *et al.*, 1984). Amides and amino acids were not determined in the present study. Free cyclitols, mostly D-pinitol and lesser amounts of *myo*-inositol, D-*chiro*-inositol and D-ononitol, accounted for 60% of the soluble carbohydrates in leaves in the current study. Sucrose and small amounts of reducing sugars were also present. In contrast, sucrose accounted for 72–85% and free cyclitols (D-pinitol, *myo*-inositol, D-*chiro*-inositol; D-ononitol was not detected) accounted for 14 (LRS) to 28% of the soluble carbohydrates unloaded from seed coats *in planta*. Rates of sucrose unloading (1–2 $\mu mol\,h^{-1}$) were similar to those previously reported (Thorne and Rainbird, 1983; Gomes *et al.*, 2005). Soybean seeds from plants expressing the mutant *stc1* and *mips* phenotypes had significantly higher sucrose concentrations than seeds from plants expressing the *Stc1* and *Mips* phenotype with normal raffinose, stachyose and phytin (Hitz *et al.*, 2002). Interestingly, sucrose unloading rates were also higher for intact seed coats on LRS plants expressing the mutant *stc1* phenotype and LRSP2 plants expressing the mutant *mips* phenotype than unloading rates for intact seed coats on CHECK plants expressing the *Stc1* and *Mips* phenotype.

Leaf pinitol concentrations increased slightly between plant growth stages R2 and R6, consistent with the pattern reported by Streeter *et al.* (2001). The pinitol-to-sucrose ratio was lower in the present study than reported by Streeter *et al.* (2001) for soybean leaves at the same node, and the ratio of pinitol to other cyclitols was lower than reported by Streeter (2001). As expected, leaf blades of all four soybean lines had similar compositions of soluble carbohydrates. Therefore, leaf tissues (or other maternal tissues) were not sampled on plants used for the seed coat cup unloading experiment. All seed coat cups were prepared on plants at the same plant growth stage and the same seed size (280–300 mg fresh weight) at mid seed fill, approximately 35 days after flowering (DAF), during the linear phase of seed dry matter accumulation. The patterns of seed fresh weight, dry weight, water content and seed growth rates were not significantly different among the four lines during this sampling period (Obendorf *et al.*, 2009). All were advanced breeding lines in related, but not isogenic, Group II maturity agronomic backgrounds. Therefore, as observed, some differences in maternal responses may be expected. Sucrose and *myo*-inositol unloading rates were highest for LRS, consistent with low raffinose and stachyose, high sucrose and high galactinol accumulations in LRS cotyledons (Obendorf *et al.*, 2009). The D-pinitol unloading rate was highest for LRSP2, consistent with the high concentrations of free D-pinitol in LRSP2 cotyledons (Obendorf *et al.*, 2009). These observations imply that differences in seed composition may be due, in part, to the supply of cyclitols unloaded from seed coats to embryos, where they may be stored as their respective cyclitol galactosides during seed maturation (Obendorf *et al.*, 2009).

Horbowicz and Obendorf (1994) and Horbowicz *et al.* (1998) proposed that galactosyl cyclitols, including fagopyritols, may function in the role of RFO in conferring desiccation tolerance to maturing seeds naturally low in raffinose and stachyose. Soybean seeds expressing the mutant *stc1* phenotype have low raffinose and low stachyose (LRS) (Sebastian *et al.*, 2000; Hitz *et al.*, 2002) but have increased galactosyl cyclitol (galactinol, fagopyritols, galactopinitols) accumulation in mature seeds, are tolerant to imbibitional chilling (Obendorf *et al.*, 2008b) and have field emergence comparable to that of seeds expressing *Stc1* and *MIPS* with normal raffinose, stachyose and phytin (Neus *et al.*, 2005). However, soybean seeds expressing the mutant *mips* phenotype have low stachyose, low phytin (LRSP1, LRSP2), and low galactosyl cyclitol accumulation in mature seeds, are sensitive to imbibitional chilling (Obendorf *et al.*, 2008b) and have lower field emergence (Meis *et al.*, 2003). Embryo tissues of buckwheat (*Fagopyrum esculentum* Moench) seeds do not accumulate raffinose (traces only) and only very small amounts of stachyose, but instead accumulate fagopyritols in

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correlation with the onset of seed desiccation tolerance (Horbowicz *et al.*, 1998). Soybean stem–leaf–pod explants (Gomes *et al.*, 2005; Obendorf *et al.*, 2008a) can provide an effective method to load exogenous substrates, including free cyclitols, through the cut stem by the transpiration stream into maternal tissues of soybean. As proof of concept, it may be possible in future experiments to demonstrate the unloading of D-*chiro*-inositol, and other substrates, from seed coat cups made surgically on soybean stem–leaf–pod explants. Indeed, increasing the supply of D-*chiro*-inositol to soybean stem–leaf–pod explants increased the accumulation of fagopyritols in mature seeds of LRS, LRSP1, LRSP2 and CHECK (Obendorf *et al.*, 2008a), but seed coat unloading on stem–leaf–pod explants has not been published.

Compositional changes in seeds expressing the mutant *stc1* phenotype or mutant *mips* phenotype in maturing embryos were not reflected in compositions of *in planta* leaf extracts, but some differences were observed *in planta* in rates of unloading compounds from seed coats to embryos expressing the mutant phenotypes. Maternally supplied cyclitols may contribute, in part, to changes in the composition of cyclitol galactosides stored in the mature seeds. Analysis of seed coat exudates may be a useful assay to survey soybean lines for increased D-*chiro*-inositol synthesis in maternal tissues, increased supply of D-*chiro*-inositol to embryos and increased accumulation of fagopyritols in mature seeds, with the potential of improving field performance for soybean seeds expressing the mutant *mips* phenotype.

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