Metabolism of Galactosylononitol in Seeds of Vigna umbellata

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In this paper, we describe the occurrence of a new galactosylononitol synthase activity (GOS) in seeds of Vigna umbellata. The enzyme catalyzed the reversible galactosyl transfer from galactinol to O-nonitol (1α-4-O-methyl-myo-inositol), yielding galactosylononitol (O-α-D-galactopyranosyl-(1 → 3)-4-O-methyl-myo-inositol) and myo-inositol. A brief characterization of the enzyme indicated a native molecular mass of 96 kDa and \( K_a \) values for galactinol and nonitol of 3.7 mM and 10.2 mM, respectively. The GOS activity could not be separated from stachyose synthase (STS) by anion-exchange and size-exclusion chromatography, suggesting that the two reactions are catalyzed by very similar enzymes or by the same enzyme. An equilibrium constant of 0.8 was determined for galactosyltransfer from galactinol to nonitol (1α). Plant cell pyranosyl-(1 → 3)-4-O-methyl-D-myo-inositol (Yasui 1980). In a previous paper, we have revised the structure of galactosylononitol

Key words: Cyclitols — Galactosylononitol synthase — Stachyose synthase — Vigna umbellata.

The development of orthodox seeds is accompanied by the accumulation of α-galactose-containing oligosaccharides in many plant species. These oligosaccharides are generally thought to play an important role in the acquisition of desiccation tolerance and, hence, seed storability by protecting macromolecular structures during storage in the dry state (Obendorf 1997). Raffinose family oligosaccharides (RFO) are among the most widespread low-molecular-mass a-galactosyltransferases. The initial step involves the formation of galactinol (O-α-D-galactopyranosyl-(1 → 3)-L-myo-inositol), which acts as galactosyl donor in RFO biosynthesis starting from sucrose (Yasui 1985). Galactinol is synthesized from UDP-galactose and myo-inositol by the action of galactinol synthase (GS; UDP-galactose:myo-inositol galactosyltransferase; EC 2.4.1.123). GS has been partially purified from leaves of cucumber (Pharr et al. 1981), squash (Webb 1982) and has been highly purified from zucchini leaves (Smith et al. 1991, Liu et al. 1995) and kidney bean cotyledons (Liu et al. 1995).

The next step in RFO biosynthesis involves the transfer of the galactosyl moiety from galactinol to sucrose. This reaction is catalyzed by raffinose synthase (RS; galactinol:sucrose galactosyltransferase; EC 2.4.1.82), which has so far only been partially purified from seeds of Vicia faba (Lehle and Tanner 1973). Another distinct galactosyltransferase, stachyose synthase (STS; galactinol:raffinose galactosyltransferase; EC 2.4.1.67), catalyzes the oligomerization of raffinose, yielding stachyose (Gaudreault and Webb 1981, Huber et al. 1990, Holthaus and Schmitz 1991a). STS also possibly synthesizes verbascose from stachyose and galactinol (Tanner et al. 1967). Higher oligomers in this series may be synthesized by a galactinol-independent galactosyltransferase activity (Bachmann et al. 1994, Bachmann and Keller 1995).

In addition to RFO, α-galactosides of inositols and, in particular, of inositol-O-methyl-ethers are found in a variety of different plant families. In seeds of important crop species such as soybean, lentil, chick pea or buckwheat, galactosides of pinitol (1β-3-O-methyl-α-myo-inositol) and chiro-inositol are major soluble components (Horbowicz and Obendorf 1994, Obendorf 1997). However, the biosynthesis of these cyclitol galactosides has not been elucidated so far.

A galactoside of ononitol (1α-4-O-methyl-myo-inositol) has been isolated from seeds of Vigna angularis and was subsequently shown to be present in 60 plant species from 21 different plant families (Yasui 1980, Yasui and Ohno 1982). The structure of this cyclitol galactoside was proposed to be O-α-D-galactopyranosyl(1 → 5)-4-O-methyl-D-myo-inositol (Yasui 1980). In a previous paper, we have revised the structure of galactosylononitol from V. angularis to O-α-D-galactopyranosyl(1 → 3)-4-O-methyl-D-myo-inositol (Richter et al. 1997). Thus, galactosylononitol represents a methylated derivative of galactinol. This finding prompted further investigations on the...
role of galactosylononitol in the carbohydrate metabolism of developing legume seeds. Here we report on the identification and partial purification of a new enzyme activity, which catalyzes the galactinol-dependent formation of galactosylononitol in seeds of *V. umbellata*. The physiological role of this galactosylononitol synthase (GOS) activity in RFO metabolism is discussed.

Materials and Methods

**Plant material**—Seeds of *Vigna umbellata* Ohwi et Ohashi (cv. VB4071) were kindly provided by the Asian Vegetable Research and Development Center, Tainan, Taiwan. Plants were grown in a growth chamber (27/20°C and 60/90% relative humidity, day/night) as previously described (Wanek and Richter 1997).

**Preparation of substrates**—D-Ononitol (lD-4-O-methyl-myo-inositol) was isolated from leaves of *V. umbellata* by chromatography on anion exchange resin and cellulose powder (Richter et al. 1992). Galactosylononitol was isolated from seeds of *V. angularis* as previously described (Richter et al. 1997). Galactinol was isolated from leaves of *Salvia officinalis* and *Melissa officinalis* according to Kuo (1992). Authentic galactinol (kindly provided by F. Keller, University of Zuerich, Switzerland) served as a standard. 

**Enzyme assays**—In the standard galactosyl transfer reaction 20 μl of enzyme extract was incubated at 30°C with 30 μl of appropriate substrate solution in gel filtration buffer. Controls were prepared by omitting one substrate. After terminating the reaction (boiling water bath for 5 min), 100 μg of a 2 : 3 (w/w) mixture of ion-exchange resins (Dowex 50–100 mesh; 50 × 8, H+ and 1 × 8, formate) and 100 μM NaCl were added to each vial. The vials were shaken at 700 rpm for 30 min and centrifuged (13,000 × g, 5 min). Aliquots of the supernatant were diluted and analyzed by HPLC-PAD as described below. All enzyme assays were optimized with respect to pH, substrate concentration and linearity with time for crude extracts.

In the case of radiolabelled substrates, the total assay volume was increased to 100 μl and the reaction stopped by the addition of 3 volumes ethanol. The assays were centrifuged and deionized. Reaction products were separated by HPLC using a flow-through liquid scintillation counter as previously described (Wanek and Richter 1995).

**Stachyose synthase (STS) and raffinose synthase (RS)**—STS activity was estimated at 30°C in a reaction mixture containing 50 mM raffinose and 10 mM galactinol in gel filtration buffer. Assays were incubated for 60 min and the formation of stachyose was measured by HPLC-PAD (DX 500, Dionex) on a CarboPac PA10 column (250 × 4 mm), with 150 mM NaOH at a flow rate of 1 ml min⁻¹ (30°C). The identity of reaction products was confirmed by a second HPLC method (Aminex HPX-87C, 300 × 7.8 mm; distilled water at 85°C, flow rate of 0.4 ml min⁻¹). A similar assay was used for the estimation of RS activity, except that raffinose was replaced by sucrose (40 mM) and assay mixtures were incubated for 3 to 4 h.
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**Galactinol synthase (GS)** was assayed at 30°C for 30 min in the presence of 20 mM myo-inositol, 5 mM UDP-galactose and 5 mM MnCl₂. An aliquot of the reaction mixture was injected onto a Carbopac PA10 column thermostated at 15°C. In this case, the column was eluted with 20 mM NaOH at a flow rate of 0.75 ml min⁻¹.

**Galactosylononitol synthase (GOS)** activity was assayed at 30°C for 60 min in a reaction mixture containing 20 mM ononitol and 10 mM galactinol. Product formation was monitored by HPLC-PAD as described for GS. For the estimation of the equilibrium constant of the reaction, 70/30 enzyme extract were mixed with 30 µl substrate solution, yielding a final concentration of (i) 10 mM ononitol and 10 mM galactinol, (ii) 5 mM each ononitol, galactinol, myo-inositol and galactosylononitol, or (iii) 10 mM myo-inositol and galactosylononitol, respectively. At intervals, aliquots were removed and product formation was monitored by HPLC-PAD on a Carbopac MA1 column with 150 mM NaOH at a flow rate of 0.4 ml min⁻¹ (20°C).

**Results**

**Galactosylononitol synthase activity**—Desalted enzyme preparations of mature seeds of *V. umbellata* were assayed for the enzymatic synthesis of galactosylononitol. Two possible galactosyl donors, UDP-galactose and galactinol were tested for the galactosylation of ononitol. Of the two substrates only galactinol served as a galactosyl donor, resulting in equimolar amounts of galactosylononitol and myo-inositol (Fig. 1). The identity of the reaction products was confirmed by two independent HPLC methods as well as by GC-MS (data not shown). Labelled galactosylononitol was found when incubating enzyme preparations with [14C-galactose]galactinol and ononitol (Table 1). However, incubation with UDP-[14C]galactose instead of galactinol only yielded substantial amounts of a labelled cyclitol galactoside, when myo-inositol was used as a galactosyl acceptor (Table 1). The crude desalted enzyme preparation of mature seeds also contained high activities of STS, while no RS activity was detected.

Anion-exchange chromatography of crude desalted extract from mature seeds resulted in a complete separation of GS from both GOS and STS activities (Fig. 2A). Pooled active GS fractions were not able to synthesize galactosylononitol from UDP-galactose and ononitol. Those fractions containing the GOS and STS activities were applied to a size-exclusion column (Fig. 2B), yielding a 24-fold purification of GOS (Table 2). However, GOS coeluted with STS activity on both columns and the ratio of activities remained unchanged for the two chromatographic steps (Table 2 and Fig. 2A, B). No RS activity was recovered from the anion-exchange column when extracts from mature seeds were used, whereas a small activity peak (<0.2 nkat, data not shown) emerging between 40 to 60 mM NaCl was detected when developing seeds (20–30 DAF) were used. However, RS activity was lost within a few hours when stored at 4°C.

The molecular mass of native GOS was estimated to be 96 kDa by size-exclusion chromatography. The pH-depend-

![Fig. 1 HPLC-PAD profiles from incubations of desalted crude extracts from mature seeds of *V. umbellata* with 10 mM galactinol and 20 mM D-ononitol for 0 hours (control, A) and 60 min (B). Metabolites were analyzed on a Carbopac MA1 column using 150 mM NaOH as eluent.](https://academic.oup.com/pcp/article-abstract/39/3/334/1925995/Metabolism-of-Galactosylononitol-in-Seeds-of-Vigna/9.1)

### Table 1 Galactosyl transfer reactions in enzyme preparations of *V. umbellata* seeds

<table>
<thead>
<tr>
<th>Donor</th>
<th>Acceptor</th>
<th>Product formed (Bq h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDP-[14C]Galactose</td>
<td>Ononitol</td>
<td>22</td>
</tr>
<tr>
<td>(14C-Galactose)galactinol</td>
<td>Ononitol</td>
<td>2,072</td>
</tr>
<tr>
<td>UDP-[14C]Galactose</td>
<td>myo-Inositol</td>
<td>5,180</td>
</tr>
<tr>
<td>Galactinol</td>
<td>myo-[3H]Inositol</td>
<td>2,923</td>
</tr>
</tbody>
</table>

In a total volume of 100 µl crude enzyme extract (0.5 mg protein) was incubated with various galactosyl donors (1 mM) and acceptors (20 mM). The specific activity of labelled substrates was 74 MBq mmol⁻¹. Reaction products were analyzed by HPLC and flow-through radioactivity detection.
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Fig. 2 Partial purification of galactosyltransferase activities from mature seeds of *V. umbellata*. (A) Anion-exchange chromatography of a crude desalted extract on Resource Q. Bound proteins were eluted with a salt gradient (50 ml) of 0–0.15 M NaCl (dashed line) in 20 mM bis-Tris propane. (B) Size-exclusion chromatography of pooled GOS/STS fractions from anion-exchange chromatography on Superdex 200 HR 10/30. ◦, GS; □, STS; ●, GOS.

ent activity curve for the galactosylation of ononitol in McIlvaine buffer showed a relatively broad maximum at pH 7.0, with half maximum activities at pH 5.5 and 8.5, respectively (data not shown). The kinetic characterization revealed a saturation response of GOS for galactinol and ononitol. Linearization by Hanes plots gave a $K_m$ value of 3.7 mM for galactinol (●, assayed with 10 mM ononitol) and at 10.2 mM for ononitol (○, assayed with 10 mM galactinol), respectively. (B) $K_m$ values were estimated at 5.9 mM for galactinol (●) and at 12.5 mM for galactosylononitol (○), respectively, in the presence of 50 mM raffinose.

and ononitol increased with the concentration of the respective cosubstrate. GOS activity did not require (divalent) cations or EDTA, but the presence of a reductant (DTT, 0.5–2 mM; data not shown).

The GOS reaction was freely reversible. The equilibrium constant was determined by incubation with varying concentrations of ononitol, myo-inositol, galactinol and galactosylononitol, as indicated (Fig. 4). Starting from either side of the reaction, an equilibrium was reached after approximately 15 h, and the concentration of galactosylononitol remained constant from then on. An equilibrium constant $K_{eq}$ of 0.8 ± 0.1 (at 30°C and pH 7.0, n = 6) was calculated for galactosylononitol synthesis from the substrate and product concentrations after 20 and 26 h. The molar sum of galactinol and galactosylononitol remained more or less unchanged throughout the experiment, indicating that hydrolytic processes were of minor importance (data not shown). A $K_{eq}$ of 0.8 at 30°C corresponds to a standard free energy ($\Delta G^\circ$) of the reaction of 0.56 kJ mol$^{-1}$.

**Table 2** Partial purification of STS and GOS activity from seeds of *Vigna umbellata*

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume (ml)</th>
<th>Total protein (mg)</th>
<th>GOS (nkat) $^*$</th>
<th>GOS (nkat mg$^{-1}$)</th>
<th>STS (nkat)</th>
<th>STS (nkat mg$^{-1}$)</th>
<th>GOS/STS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>12.0</td>
<td>128.5</td>
<td>19.4</td>
<td>0.15</td>
<td>14.1</td>
<td>0.11</td>
<td>1.38</td>
</tr>
<tr>
<td>Resource Q</td>
<td>4.0</td>
<td>8.6</td>
<td>10.3</td>
<td>1.21</td>
<td>9.9</td>
<td>1.16</td>
<td>1.04</td>
</tr>
<tr>
<td>Superdex 200</td>
<td>1.2</td>
<td>1.2</td>
<td>4.3</td>
<td>3.66$^b$</td>
<td>4.0</td>
<td>3.38</td>
<td>1.08</td>
</tr>
</tbody>
</table>

$^*$ Total amount of activity.

$^b$ This value corresponds to a 24-fold purification.
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Fig. 4 Equilibrium of the GOS reaction. Crude desalted extracts were incubated at 30°C with 10 mM ononitol and 10 mM galactinol (●), 5 mM of each ononitol, galactinol, myo-inositol and galactosylononitol (○), and 10 mM myo-inositol and galactosylononitol (□), respectively. Aliquots were removed at times indicated and product formation monitored by HPLC-PAD as described in Material and Methods.

and [14C-galactose]galactinol yielded labelled stachyose (Fig. 5A). Additionally this preparation was also able to utilize [14C-galactose]galactosylononitol for the galactosylation of raffinose, although at a lower rate as galactinol (Fig. 5B). The kinetic characterization of STS revealed \( K_m \) values of 5.9 mM for galactinol and 12.5 mM for galactosylononitol (both assayed with 50 mM raffinose; Fig. 3B) and 19.6 mM for raffinose (assayed with 10 mM galactinol, data not shown). As already found for GOS activity, the \( K_m \) value for galactinol increased in the presence of high raffinose concentration. STS also exhibited the same pH optimum (pH 7.0) and native molecular mass as GOS.

Soluble carbohydrates and cyclitol galactosides during seed development—*V. umbellata* seeds reached maturity 40 DAF. The seed moisture concentration declined from 83.1% (10 DAF) to 12.3% in dry seeds (40 DAF), while seed dry weight increased to 70.3 mg seed\(^{-1}\) (Fig. 6). During the first 18 DAF myo-inositol and ononitol increased to about 0.5 and 0.6 \( \mu \text{mol seed}^{-1} \) and declined thereafter to levels of about 0.2 and 0.4 \( \mu \text{mol seed}^{-1} \), respectively (Fig. 7A). Galactinol and galactosylononitol were not de-
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by guest

Protected until 16 DAP. Both cyclitol galactosides accumulated rapidly from 16 to 22 DAF, after which they remained fairly constant through 26 DAF. During the late stages of development, galactinol declined to almost undetectable levels (Fig. 7B). Maximum galactitol concentration calculated on a plant water basis was 4.1 mmol liter$^{-1}$ (26 DAF, data not shown). In contrast, galactosylononitol content increased substantially until seed maturity (0.5 to 1.2 mmol seed$^{-1}$, 24 to 40 DAF) (Fig. 7B). Calculated on a plant water basis galactosylononitol increased from 7.5 to 67.7 mmol liter$^{-1}$ (24 to 31 DAF), reaching a concentration of 124.8 mmol liter$^{-1}$ in mature seeds (40 DAF).

RFO were almost undetectable until 22 DAF, but accumulated quickly during the late stages of seed maturation. As early as 24 DAF stachyose content (1.5 µmol seed$^{-1}$) exceeded that of galactosylononitol threefold (Fig. 7C). Sucrose, the dominant carbohydrate until 22 DAF, sharply decreased thereafter (Fig. 7C). By seed maturity stachyose was the predominant sugar (3.6 µmol seed$^{-1}$), followed by sucrose (1.5 µmol seed$^{-1}$), galactosylononitol and raffinose. In addition, low amounts of a digalactosylononitol were present in the late stages of seed development (<0.1 µmol seed$^{-1}$, data not shown). Cyclitol galactosides and RFO were not detected in leaves and pod walls (data not shown).

**Enzyme activities in developing seeds—**Low or undetectable levels of GS, STS and GOS activity were observed during early stages of seed development (Fig. 7D–F). Beginning about 18 DAF, activities of these enzymes increased dramatically. GS peaked at 28 to 31 DAF (1.6 nkat seed$^{-1}$) and decreased to about 50% of the maximum activity in dry seeds (Fig. 7D). However, STS and GOS activity continued to increase, reaching 0.2 and 0.3 nkat seed$^{-1}$, respectively (Fig. 7E, F). In contrast to the time-dependent pattern of galactosylononitol and stachyose accumulation, GOS activity exactly paralleled that of STS throughout seed development. Both activities remained high in dry seeds (approximately 0.1 to 0.2 nkat seed$^{-1}$ after one year). From 22 to 31 DAF traces of RS activity (<5 pkat seed$^{-1}$) were detected (data not shown).

**Discussion**

Galactosylononitol represents a methylated derivative of galactinol. Due to this close structural relationship, a similar biosynthetic pathway might be possible (Richter et al. 1997). However, all attempts to demonstrate enzymatic synthesis of galactosylononitol analogous to that of galactinol by transgalactosylation from UDP-galactose were not successful. GS, partially purified from *V. umbellata* could not utilize ononitol as galactosyl acceptor. This is in agreement with the enzyme from pea seeds, which was not able to utilize the ononitol isomer pinitol (Frydman and Neufeld 1963). However, GS from zucchini squash leaves and pea seeds accepted several myo-inositol isomers (L-chiro-, D-chiro-, scylo- and epi-inositol) as substrates (Frydman and Neufeld 1963, Smith et al. 1991).

Instead of galactosyl transfer via the nucleotide pathway the biosynthesis of galactosylononitol in *V. umbellata* seeds was found to proceed via galactosyl transfer from galactinol to ononitol (Fig. 1). The corresponding enzyme activity is a new observation and was formally termed galactinol: ononitol galactosyltransferase or galactosylononitol synthase (GOS). Although GOS activity is clearly distinct from RS there are several pieces of evidence suggesting that STS and GOS activities are catalyzed either by very similar enzymes or by the same enzyme. Firstly, STS and GOS activity copurified on anion-exchange and size-exclusion chromatography (Fig. 2A, B) with a constant activity ratio (Table 2). Secondly, the two activities showed the same pH optimum and similar kinetic properties (Fig. 3). Finally, the pattern of activity of GOS and STS were identical during seed development (Fig. 7). Moreover, the GOS activity closely resembles a galactosyl exchange reaction between galactinol and myo-inositol catalyzed by STS in seeds of *Phaseolus vulgaris* and leaves of *Cucurbita pepo* (Tanner and Kandler 1968, Gaudreault and Webb 1981), in that the galactosyl residue of galactinol is transferred to methylated myo-inositol (ononitol) instead of myo-inositol. The exchange reaction was also detected in *V. umbellata* (Table 1).

While the above results clearly demonstrate that galactinol is involved in the biosynthesis of galactosylononitol, it cannot be ruled out that other biosynthetic pathways contribute to the overall synthesis of this cyclitol galactoside. By summing up the average GOS activity between consecutive harvests from 16 to 31 DAF (Fig. 7E) a total capacity of 2,130 µmol (g DW)$^{-1}$ for galactosylononitol synthesis was estimated. Thus developing seeds contain, on average, the enzymatic capacity to synthesize the amount of galactosylononitol actually present in mature seeds (17.5 µmol (g DW)$^{-1}$) within less than 3 h.

The physiological role of cyclitol galactosides is still not fully understood (Obendorf 1997). It has been suggested that these compounds may act as galactosyl donors in RFO biosynthesis analogous to galactinol (Beveridge et al. 1977, Dey 1985). The kinetic characterization of the reversible GOS reaction allows for the first time an estimation of the galactosyl transfer potential of a methylated cyclitol galactoside. The fairly low standard free energy (0.56 kJ mol$^{-1}$) estimated for the galactosyl transfer reaction from galactinol to ononitol yielding galactosylononitol and myo-inositol (Fig. 4) suggests that the group transfer potential of galactosylononitol is similar to that of galactinol and sufficient for galactosylation of RFO. For the synthesis of stachyose from galactinol and raffinose, an equilibrium constant of approximately 4.0 (at 32°C) has been determined (Tanner and Kandler 1968). This corresponds to a negative free energy of hydrolysis of galactinol,
approximately 3.5 kJ mol⁻¹ higher than that for hydrolysis of the terminal galactosyl linkage of stachyose. STS partially purified from *V. umbellata* seeds was indeed able to utilize galactosylononitol instead of galactinol for the biosynthesis of stachyose from raffinose (Fig. 5). The concentration of galactosylononitol during the late stages of seed development dramatically exceeded that of galactinol, suggesting favourable conditions for stachyose synthesis to proceed via galactosylononitol, although kinetic data indicated that galactosylononitol was a less effective galactosyl donor compared to galactinol.

Galactosylononitol may also act as galactosyl donor in the formation of other RFO. However, only low and instable activity of RS was extractable from developing seeds of *V. umbellata*, preventing a characterization of this enzyme with respect to cyclitol galactoside specificity. RS activity extracted from soybean, kidney bean and squash was also reported to be particularly instable (Gaudreault and Webb 1981, Castillo et al. 1990).

The proposed pathways of galactosylononitol synthesis and utilization are consistent with the developmental patterns of enzyme activities and carbohydrates in seeds of *V. umbellata* (Fig. 7). Similar patterns of pool sizes were observed for a galactosylpinitol, galactinol and RFO during seed development of *Leucaena leucocephala* (Chien et al. 1996). In that paper galactopinitol was proposed to be an intermediate in the biosynthesis of higher pinitol galactoside oligomers. However, as already mentioned, higher oligomers of galactosylononitol were only present in minute amounts in *V. umbellata*.

The results presented above suggest that the accumulation of galactosylononitol in *V. umbellata* seeds may play a role in facilitating RFO metabolism. In many plant species sucrose is converted into RFO during seed development (Obendorf 1997). During early stages of seed development of *V. umbellata* additional galactosyl residues could be metabolized into galactosylononitol prior to raffinose formation, utilizing the ononitol which is already present in the seeds as an intermediate. Subsequently, galactosylononitol may be used for the synthesis of RFO. Since the GOS reaction is fully reversible with an equilibrium constant close to one, however, galactosylononitol may also resupply the galactinol pool in the late stages of seed development, depending on actual subcellular concentration of substrates and products. Several authors favoured a cytoplasmic compartmentation of GS, STS, myo-inositol and galactinol in leaves and tubers (Keller 1992, Holthaus and Schmitz 1991b, Bachmann and Keller 1995). Sucrose, raffinose and stachyose, in contrast, were also present in protein bodies from lupine and cotton seeds (Muller and Jacks 1983, Plant and Moore 1983), suggesting that total extractable carbohydrate levels may not necessarily reflect in vivo concentrations. Thus, clarification of enzyme, substrate and product localization will be essential for a deeper understanding of in vivo function of galactosylononitol in seed oligosaccharide metabolism. On the other hand, accumulation of galactosylononitol at late stages of seed development may also contribute to the acquisition of desiccation tolerance, as has already been proposed for other cyclitol galactosides (Horbowicz and Obendorf 1994, Chien et al. 1996, Obendorf 1997).

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**References**


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