A COMPARISON OF SOLUBLE SUGAR ACCUMULATION IN ZYGOTIC AND SOMATIC PEA EMBRYOS

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Key words: desiccation, somatic embryogenesis, somatic embryos, soluble sugars.

Abbreviations: DAF – day after flowering; DW – dry weight; FW – fresh weight; RFOs – raffinose family oligosaccharides

Abstract

This study compares the soluble sugar content of zygotic and somatic pea embryos. It was noted that mature somatic embryos differed from zygotic embryos with respect to carbohydrate composition. Mature zygotic pea embryos contained glucose, myo-inositol, sucrose, maltose, galactinol, galactosyl-cyclitols, raffinose, stachyose and verbascose. The presence of maltose, galactosyl-cyclitols, stachyose and verbascose was not determined in somatic embryos, and their total soluble sugar content was below that of zygotic embryos. High sucrose levels in somatic embryos most probably resulted from the presence of sucrose in the growth medium. Monocotyledonous and irregular somatic embryos were characterized by a different sugar profile than regularly shaped somatic embryos and seeds.
A b s t r a k t

W pracy porównano zawartość węglowodanów rozpuszczalnych w zygotycznych i somatycznych zarodkach grochu. Odnotowano, iż skład węglowodanowy dojrzałych zarodków somatycznych jest zmodyfikowany w porównaniu z zarodkami zygotycznymi. W dojrzałych zarodkach zygotycznych grochu występowały glukoza, myo-inositol, sacharoza, maltosa, galaktinol, galaktozylo-cykliotole, rafinoza, stachioza i werbaskoza. W zarodkach somatycznych nie wykryto maltozy, galaktozylo-cykliotoli, stachiozy i werbaskozy, a całkowita zawartość cukrów rozpuszczalnych była niższa niż w zarodkach zygotycznych. Wysoka zawartość sacharozy w zarodkach somatycznych była prawdopodobnie skutkiem jej obecności w podłożu wzrostowym. Jednoliścieniowe oraz nieregularne zarodki somatyczne wykazywały odmienny profil cukrowy niż prawidłowe zarodki somatyczne i nasiona.

I n t r o d u c t i o n

Storage materials are accumulated at the final stages of embryo matura-
tion. The seeds and embryos of legumes accumulate different types of soluble sugars (GÓRECKI et al. 2000). Maturing zygotic seeds store sucrose, raffinose family oligosaccharides (RFOs), cyclitols and galactosyl-cyclitols (GÓRECKI and OBENDORF 1997), and their starch content is subject to fluctuation (GÓRECKI et al. 2000).

Starch is one of the main reserve sugars in the pea. It is accumulated during the development of zygotic embryos, but this process is not always observed during somatic embryogenesis. Starch accumulation in somatic embryo cells was investigated by LOISEAU et al. (1998). There is a general scarcity of studies exploring the accumulation of soluble sugars in somatic embryos (e.g. GÓRECKI et al. 2000), therefore, the objective of this experiment was to compare the accumulation of soluble sugars during zygotic and somatic embryogenesis of the pea, which significantly affects the process of embryo desiccation.

M a t e r i a l s a n d M e t h o d s

P l a n t m a t e r i a l

The experimental material comprised the seeds of pea var. Oskar and HM-6 supplied by AGRITECH Ltd. of the Czech Republic. To obtain zygotic embryos, seeds were placed in pots filled with compost soil and sand (4:1, v/v). The seeds were regularly watered with tap water. The moisture content of the substrate was maintained at 60–70%. The Florovit fertilizer was applied three times: at the stage of five leaves, at the beginning of flowering and at fruiting. The first maturing pods were harvested 10 days after flowering (DAF), and then every
4 days until full maturity. The collected seeds’ fresh weight, dry weight, vigor and viability were determined.

The material for culturing somatic embryos was excised from four-day-old, etiolated, axenically raised seedlings. To obtain axenically grown seedlings, seeds were surface sterilized in a 5% aqueous solution of Chloramine B for 15 minutes, followed by three washes with sterile distilled water. Disinfected seeds were placed in sterile tubes (25 ml capacity) containing moist cotton wool. After germination (in darkness at 25–26°C for four days), shoot apices were excised from seedlings using a dissecting microscope, and they were placed on the induction medium.

Explants were subjected to 14-day induction on the basal medium described by Griga (1998) that contained MS salts (MURASHIGE and SKOOG 1962), Gamborg B5 vitamins (GAMBORG et al. 1968), 3% sucrose and 2.5 μM picloram. After induction, all cultures were transferred to the differentiation medium – a basal medium without phytohormones. The cultures were kept in a growth chamber under the 16:8 photoperiod (light:darkness) and at temperatures of 23–24°C during the day and 19–20°C at night.

**Determination of the soluble sugar content of zygotic and somatic embryos**

Soluble sugars were extracted by the modified method proposed by Górecki et al. (1997). The pea flour obtained from ground pea seeds or parts thereof (around 50 mg) was combined with 100 μg of internal standard (xylitol) and 800 μl of 70% ethanol solution, and it was placed in a water bath with a temperature of 60–65°C for 35 minutes. The samples were cooled to room temperature and centrifuged at 22 000 g for 30 minutes. The supernatant was transferred through Dovex 50Wx8-100 and Dover 2x8 ion exchange columns. After centrifuging (22 000 g; 10 min; 20°C), 200 μl of the supernatant was transferred to chromatographic vials and dried in a vacuum centrifuge. Dried samples were stored in a desiccator over silica gel (Silica Gel Blue, Fluka). Soluble sugars were extracted from live somatic embryos using the same procedure.

Prior to chromatographic separation, the sugars were dissolved in a mixture of TMSi (N-trimethyl-silylimidazole) and pyridine at a temperature of 90°C for 60 minutes. TMS-derivatives were separated using a capillary column in the GC-2010 gas chromatograph (SHIMADZU). The sugars were separated in a temperature gradient of 150 to 325°C. The carrier gas was helium, applied at a flow rate of 1.25 cm²/min. The sugars were identified by comparing their retention times (total and relative) against commercially available standards.
The number of sugars was determined based on simple regressions calculated for changes in the ratio of the sugar surface area to the surface area of the internal standard.

Results and Discussion

In legumes, the accumulation of soluble sugars during seed maturation is related to the development of desiccation resistance. This effect is attributed mainly to the accumulation of sucrose, raffinose, stachyose and verbascose. The stachyose + raffinose : sucrose ratio approximates 1 when seeds become resistant to dessication and reach full maturity (BAILLY et al. 2001). The presence of fructose, glucose, myo-inositol, sucrose, maltose, galactinol, galactosyl-cyclitol, raffinose, stachyose and verbascose was determined in maturing zygotic embryos. Changes in the carbohydrate composition of zygotic embryos were observed during maturation (Figure 1). The embryos of mature pea seeds contained mostly sucrose, galactinol, raffinose, verbascose and stachyose as well as trace amounts of fructose, glucose, maltose and myo-inositol (Table 1). Somatic pea embryos contained fructose, glucose, myo-inositol, sucrose, raffinose and galactinol. The presence of maltose, galactosyl-cyclitol, stachyose and verbascose was not found, and the total soluble sugar content of somatic embryos was several times lower in comparison with zygotic embryos (Table 2). The sugar profile of normal (dicotyledonous) somatic embryos was most similar to that of several-days-old zygotic embryos. Sucrose was the main soluble sugar accumulated by somatic embryos, and it had more than a 75% share of all soluble sugars in the somatic embryos of var. HM-6. In var. Oskar, sucrose levels reached 65.2% in dicotyledonous embryos and 52.4% in monocotyledonous embryos. Similar quantities of sucrose were observed during seed maturation, ranging from 27.1% to 89.7% in var. HM-6, and from 17.6% to 90.5% in var. Oskar (Table 1). The noted results are consistent with the findings of Blöchl et al. (2005) who studied the somatic embryos of alfalfa. In zygotic embryos, an increase in sucrose levels is accompanied by the onset of maturation which is characterized by the rapid growth of fresh and dry weight, a drop in water content, the emergence of starch granules and albuminous substances (e.g. SANCHEZ-ROMERO et al. 2002). The rise in sucrose levels in mature somatic embryos is generally attributed to intensive embryo metabolism (IRAQUI et al. 2005), sucrose conversion to oligosaccharides (LiN et al. 1998) and direct sucrose uptake from the culture medium by explant and embryo cell enzymes without prior hydrolysis (ŻUR et al. 2002, IRAQUI et al. 2005).
Fig. 1. The soluble sugars concentration [µg mg⁻¹] in pea seeds, embryo axes, cotyledons and seed coats (measured to one milligram of fresh and dry weight). DW – dry weight, FW – fresh weight. 

- a – fructose,
- b – glucose,
- c – myo-inositol,
- d – sucrose,
- e – maltose,
- f – galactinol,
- g – raffinose,
- h – stachyose,
- i – verbascose.

* the seeds from 10 and 14 DAF were not part to embryo axes, cotyledons and seed coats.
Soluble sugar accumulation in pea seeds

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Oskar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 DAF</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.108 (0.632)</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.205 (1.232)</td>
</tr>
<tr>
<td><strong>Myo-inositol</strong></td>
<td>1.657 (9.847)</td>
</tr>
<tr>
<td>Sucrose</td>
<td>25.253 (147.900)</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.411 (2.403)</td>
</tr>
<tr>
<td>Galactionol</td>
<td>0.033 (0.191)</td>
</tr>
<tr>
<td>Gal-cyclitols</td>
<td>0.100 (0.587)</td>
</tr>
<tr>
<td>Raffinose</td>
<td>0.119 (0.697)</td>
</tr>
<tr>
<td>Stachyose</td>
<td>0</td>
</tr>
<tr>
<td>Verbasose</td>
<td>0</td>
</tr>
<tr>
<td>Raffinose/sucrose</td>
<td>0.005 (0.005)</td>
</tr>
<tr>
<td><strong>Total soluble sugar accumulation</strong></td>
<td>27.886 (163.488)</td>
</tr>
</tbody>
</table>

In the seeds of many plant species, the highest monosaccharide concentrations are observed at the early stages of embryo development, while trace amounts of monosaccharides are found in mature seeds (Sánchez-Romero et al. 2002). The results of the existing research suggest that mature pea seeds contain no reducing sugars (Górecki and Obendorf 1997), yet certain varieties demonstrate small quantities of fructose and glucose (Górecki et al. 2000). The results of this study confirm the above observations. Monosaccharides were not found in the mature seeds of var. HM-6, while trace amounts of reducing sugars were determined in Oskar seeds (around 0.07%) – Table 1. Fluctuations in total glucose and fructose concentrations were also noted during the maturation of zygotic embryos (Figure 1). The seeds of other legume species, such as lupine, are marked by low glucose levels (Górecki et al. 1997). In normal, dicotyledonous embryos of var. Oskar, reducing sugars had an estimated 8.70% share, whereas in var. HM-6, the total concentrations of fructose and glucose accounted for 9.46% of all determined sugars (Table 2). In irregular, monocotyledonous embryos of var. Oskar, monosaccharide concentrations were very high at 43% and 100% of total soluble sugars. The above can probably be attributed to the physiological immaturity of embryos.
<table>
<thead>
<tr>
<th>Carbohydrates</th>
<th>Oskar</th>
<th>2-cotyledonous</th>
<th>1-cotyledonous</th>
<th>irregular</th>
<th>1-cotyledonous</th>
<th>irregular</th>
<th>HM-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose</td>
<td>0.246 ± 0.015</td>
<td>0.3 ± 0.015</td>
<td>0.0</td>
<td>0.247 ± 0.002</td>
<td>0.361 ± 0.009</td>
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<tr>
<td>Glucose</td>
<td>0.718 ± 0.050</td>
<td>0.279 ± 0.001</td>
<td>0.0</td>
<td>0.412 ± 0.011</td>
<td>0.545 ± 0.017</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myo-inositol</td>
<td>1.589 ± 0.030</td>
<td>0.0</td>
<td>0.0</td>
<td>0.950 ± 0.011</td>
<td>1.030 ± 0.007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>7.221 ± 0.017</td>
<td>1.018 ± 0.001</td>
<td>0.0</td>
<td>5.644 ± 0.002</td>
<td>7.286 ± 0.031</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galactitol</td>
<td>0.230 ± 0.017</td>
<td>0.0</td>
<td>0.0</td>
<td>0.219 ± 0.011</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raffinose</td>
<td>0.15</td>
<td></td>
<td></td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total soluble sugar accumulation</td>
<td>11.075 ± 0.008</td>
<td>1.943 ± 0.001</td>
<td>0.0</td>
<td>6.501 ± 0.147</td>
<td>9.222 ± 0.048</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Myo-inositol was found in both the normal and abnormal somatic embryos of var. HM-6, whereas in var. Oskar it was determined solely in dicotyledonous embryos (Table 2). In comparison with zygotic embryos (1.64% to 7.12% soluble sugars in var. HM-6, and 1.31% to 5.94% in var. Oskar), regularly shaped somatic embryos were characterized by elevated levels of myo-inositol (12.62% and 14.35% soluble sugars, respectively). Despite relatively high myo-inositol concentrations in somatic embryos, galactosyl derivatives of this compound were not observed in noticeable amounts (galactinol was found solely in the dicotyledonous embryos of var. Oskar). According to GÓRECKI et al. (2000), the zygotic embryos of pea seeds contain 1.65% to 3.08% inositol and other cyclitols. In a study of maturing soybean seeds, Obendorf et al. (1998) observed that myo-inositol concentrations decrease with an increase in galactinol levels. Higher myo-inositol concentrations were observed in in vitro-matured zygotic embryos. This could suggest that elevated myo-inositol levels in somatic embryos are a characteristic feature of in vitro cultures. ŻUR et al. (2002) reported that the active uptake of myo-inositol from the medium by explant cells increased during the initiation of organogenesis in rapeseed cultures. An increased uptake of myo-inositol could testify to its key role in plant metabolism, such as signal transduction and resistance to stress (LOEWUS and MURTHY 2000).

Raffinose was the only raffinose family oligosaccharide detected in the dicotyledonous somatic embryos of both studied varieties. Raffinose content in var. Oskar and HM-6 was 9.67% and 2.91%, respectively. Raffinose levels in seeds also varied between the varieties (Table 1). In mature zygotic embryos, the raffinose to sucrose ratio was determined at 0.38 and 0.83 in var. HM-6 and Oskar, respectively. The values of the above ratio were very low in somatic embryos, reaching around 0.15 in var. Oskar and 0.04 in var. HM-6. Sucrose and raffinose also had a varied share of the total soluble sugar content. In mature zygotic embryos, sucrose proportions were determined at 0.28 in var. HM-6 and 0.24 in var. Oskar. In HM-6 somatic embryos, the sucrose to soluble sugars ratio reached 0.75, 0.77 and 0.79 for dicotyledonous, monocotyledonous and irregular embryos, respectively. In Oskar somatic embryos, the above ratio was determined at 0.65, 0.52 and 0 for dicotyledonous, monocotyledonous and irregular embryos, respectively. The raffinose to soluble sugars ratio reached 0.09 and 0.13 in mature zygotic embryos, and 0.03 and 0.09 in dicotyledonous embryos of var. HM-6 and Oskar, respectively. The absence of RFOs in monocotyledonous and irregular embryos could result from low myo-inositol levels (LOEWUS and MURTHY 2000). The raffinose to sucrose ratio in the above embryos was also low, suggesting that the produced somatic embryos did not reach physiological maturity.
Conclusions

The results of this study indicate that somatic embryos contain high levels of soluble sugars, such as sucrose, myo-inositol, raffinose and monosaccharides. Contrary to zygotic embryos, the presence of stachyose and verbascose was not noted in somatic embryos.

High sucrose levels in somatic embryos probably resulted from the presence of sucrose in the culture medium.

Monocotyledonous somatic embryos and irregular embryos were characterized by a different sugar profile than normal somatic embryos and seeds.

It can be concluded that selected developmental defects observed in somatic embryos (number of cotyledons) could be attributed to growing conditions and the accumulation of monosaccharides and raffinose family oligosaccharides.

Translated by Aleksandra Poprawska

References


