PHYSIOLOGICAL RESPONSES TO HIGH EXOGENOUS SUCROSE IN TUBER INDUCTION OF POTATO IN VITRO

Xueqin HE*, Meilian MENG

Inner Mongolia Agricultural University, Agronomy Faculty, Hohhot, CHINA
*Corresponding author: xueqinhe2013@163.com

Received: 31.11.2015

ABSTRACT

Microtubers are valuable tools to be used in the researches of potato. Among the factors sucrose is the most critical stimulus for potato microtuber production in vitro. The aim of our study was to define whether only sucrose was enough to induce the tuber and how it impacted on physiological responses in potato in vitro. Among sucrose concentrations chosen 80 and 100g/l sucrose could induce maximum tubers in three cultivars of Solanum tuberosum L., Atlantic, Desiree and 851 in vitro at 22 °C under 16-h photoperiod. Meanwhile tuber induction percentage showed no difference in MS with 80 and 100g/l sucrose among 0, 16 and 24-h photoperiod and cuttings with no nodes induced no tubers in cv. Atlantic. Therefore, 80 or 100g/l of exogenous sucrose concentrations and cuttings with node were essential to in vitro tuber induction in potato. The endogenous sucrose content and acid invertase in induced cuttings were higher than corresponding tubers and dramatically increased with the increase of sucrose concentration in MS. Starch content was completely opposite to endogenous sucrose content and decreased with the increase of sucrose concentration in MS. Sucrose synthase (Susy) and sucrose phosphate synthase (SPS) accumulated only in tubers, but no significant differences of Susy and SPS were between 80g/l and 100g/l sucrose. In conclusion, high levels of exogenous sucrose resulted in a dramatic high gradient of endogenous sucrose content between inducing cuttings and the corresponding tubers, which was beneficial to trigger differential activity expression of sugar metabolizing enzymes and to accumulate starch in tubers in vitro.

Keywords: Microtuber; potato; sucrose; sugar metabolic enzymes

INTRODUCTION

Microtubers were used in seed potato (Solanum tuberosum L.) production (Öztürk & Yildirim, 2010) and potato germplasm conservation (Lizarra, 1989), as well as in analyzing of physiological and biochemical metabolism (Cenzano et al., 2006, 2008; Raíces et al., 2003a) and gene expression associated with tuber formation and growth (Gargantini et al., 2009; Raíces et al., 2003c). It also served as a source for studies of genetic transformation (Gargantini et al., 2009; Si et al., 2008).

Since Barker (1953) first used etiolated sprouts to induce potato tubers in a medium containing 80g/l sucrose, high sucrose ranging from 60 to 90g/l and different explants including apical/uppermost nodal sections of the shoot and nodal segments/cuttings were used to induce in vitro tubers under a short photoperiod from 8 to 10h or in the dark (Altindal & Karadogan, 2010; Aslam et al., 2011; Dhital & Lim, 2012; Hoque 2010; Hussain et al., 2006; Motallebi-Azar et al., 2013; Servet et al., 2000). Wang & Hu (1982) and Ewing & Struik (1992) considered sucrose may be the most critical stimulus for potato microtuber production in vitro.

During the stages of potato tuber formation developing tubers become the largest sinks accompanying increased cell division and expansion and a massive deposition of starch (Gargantini et al., 2009; Prat et al., 1990; Visser et al., 1994). Khuri & Moorby (1995) found that the translocation and usage of sucrose was faster than the other sugars in the tubering plantlet in vitro. A large proportion of sucrose taken up into sink-tissue was converted to starch in rapidly growing potato tubers (Oparka et al., 1990; Wright & Oparka, 1989). The accumulation of starch (Visser et al., 1994) and the quantity and composition of soluble sugars (Davies, 1984) associated with a tuber undergo significant changes. High sucrose levels increased Susy in the potato in culture medium (Sowokinos & Varns, 1992). Sucrose can be hydrolyzed by either invertases or Susy in most plants (Koch, 2004; Kruger 1990). The reaction catalyzed by invertase is highly irreversible and by Susy is freely reversible in the direction of sucrose degradation (Avigad 1982; Geigenberger & Stitt, 1993; Kruger 1990; Morell & Copeland, 1985).
Sucrose was considered to be the most critical stimulus for potato microtuber production in vitro (Ewing & Struik, 1992; Wang & Hu, 1982). The objective of the present work was to determine whether sucrose was the only essential factor for tuber induction and how it influenced sugar metabolic activities in potatoes in vitro. Our results will be facilitated to develop strategies for optimized crop management for high microtuber yield and to save cost in vitro and in the green house.

MATERIALS AND METHODS

Plant material

Stem cuttings were taken from 4 week microplants of potato (Solanum tuberosum L.) cultivars such as Atlantic, Desiree and 851 in vitro at 22 °C under 16-h photoperiod (124 µmol /m² s¹). All stem cuttings were prepared from middle nodes of the microplants.

In vitro Tuberization

Two-node cuttings of potato cultivars were placed vertically on MS (Murashige & Skoog, 1962) medium containing 50, 60, 80, 100 and 120 g/l sucrose at 22 °C under 16-h photoperiod. From the 7th day induced tubers were recorded every 2 days until the 19th day. After determining the optimal sucrose concentration in MS the effects of photoperiods (0, 16 and 24 h) and node numbers of the cuttings (0, 1 and 2 nodes) were studied, respectively. Tuber induction percentage (TIP) was calculated as a percentage on the basis of total induced cuttings.

Induced cuttings and corresponding tubers were separately harvested in MS with 80g/l and 100g/l sucrose at 22 °C under 16-h photoperiod on the 19th day and were used in the following studies.

Content of sucrose and starch

For determining sucrose and starch content 100mg of fresh tissue were ground and extracted in 80% (v/v) ethanol. The extract was centrifuged at 4000g for 5 min and its supernatant fraction was evaporated, and then the residues were suspended in purified water and stored at -20 °C to use. The pellets free from sugar were extracted with 80% ethanol again, and then were dried and stored for starch extraction.

Sucrose was determined according to Wang (2000) with a 2.3ml reaction mixture including 0.1ml of extract, 0.2mM NaOH, 6.2M HCl and 2 mM o-Dihydroxybenzene. Readings were made at 480nm. Sucrose content was estimated using a sucrose calibration curve.

Starch was based on Hovenkamp-Hermelink et al. (1988) with modification. Starch was extracted from the dried sugar-free residue with 5M Ca(NO₃)₂ at 100 °C for 5 min, and the extract centrifuged at 3000g for 5 min. 1-KI staining was achieved by mixing 5ml of supernatant and 5ml of 20mM KI and 6.5mM I₂. Absorbencies were measured immediately after mixing at 620nm. Starch content was estimated using a glucose calibration curve.

Sugar metabolism enzyme

One hundred mg of fresh tissue was ground at 4 °C and extracted with an extraction buffer including 50mM HEPES-KOH (pH 7.4), 5mM MgCl₂, 1mM EDTA, 5mM DTT and 2% insoluble PVP. The extract was centrifuged at 16,000g at 4 °C for 10 min and the supernatant was used in enzyme activities analysis. The reaction with heat-inactivated enzyme preparation was used as control.

Soluble acid invertase (E.C. 3.2.1.26) was measured based on Geigenberger & Stitt (1993) with modification. The reaction mixture contained 0.1ml supernatant, 100mM sucrose, 50mM sodium citrate (pH 5.0), and 50mM KCl. Glucose formed was determined as described above.

Sucrose synthase (Susy: E.C.2.4.1.13) and sucrose-6-phosphate synthase (SPS: E.C.2.4.1.14) were measured according to Zhu et al. (1997). The Susy assay solution was 20mM fructose, 20mM UDP-glucose, 1mM MgCl₂ and 50mM HEPES-KOH (pH 7.0), and the SPS assay solution included 100mM Hepes (pH 7.5), 20mM glucose-6-phosphate, 20mM fructose-6-phosphate, 20mM UDP-glucose, 1mM MgCl₂, and 1mM EDTA. Sucrose produced by these reactions was assayed using the anthrone assay.

Protein contents were determined according to the Bradford (1976) method.

Statistical analysis

Every treatment was repeated 3 times and one replicate included two 100ml-flasks with 10 cuttings per flask. This experiment was repeated twice in laboratory of Inner Mongolian Agricultural University, Hohhot in 2012.

Completely randomized blocks statistical design and SAS9.0 were used for comparing the means. The significance of differences between the variables was taken according to LSD test at P≤0.05.

RESULTS

Defining optimal concentration of exogenous sucrose on tuber induction in vitro

Different concentrations of exogenous sucrose in MS had different TIPs in Solanum tuberosum L. cv. Atlantic. Cuttings dried early and no tuber appeared in MS with 120g/l sucrose. The TIPs from MS with 80 and 100g/l sucrose were much higher than 50 and 60g/l sucrose, but the TIPs between 80 and 100g/l sucrose had no significant difference during the induction period except on the 11th day (Figure 1). Although the TIP, diameter and fresh weight of induced tubers by 80g/l sucrose were higher than those by 100g/l sucrose on the 19th day, no significance was detected (Table 1). The same results were obtained for the same experiments in potato cultivars, 851 and Desiree and the average tuber number per cutting of 80g/l and 100g/l sucrose were listed in Table 2. The results suggested that 80g/l and 100g/l sucrose had the same effect and were better than 50 g/l and 60 g/l sucrose on tuber induction in different potato cultivars in vitro.
**Figure 1.** Tuber induction percentage in MS with different concentrations of exogenous sucrose in *Solanum tuberosum* L. cv. Atlantic (* indicate differences within the group at 0.05 level).

**Table 1.** Effect of 80g/l and 100g/l sucrose on induced tubers in *Solanum tuberosum* L. cv. Atlantic in vitro

<table>
<thead>
<tr>
<th>Tubers caliber range (mm)</th>
<th>Average tuber number/cutting</th>
<th>Fresh weight /tuber (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 g/l</td>
<td>2.002-6.010</td>
<td>0.683±0.153</td>
</tr>
<tr>
<td>100 g/l</td>
<td>2.000-5.014</td>
<td>0.667±0.104</td>
</tr>
</tbody>
</table>

**Table 2.** Average induced tubers of *Solanum tuberosum* L. cv., 851 and Desiree in MS with 80g/l and 100g/l sucrose

<table>
<thead>
<tr>
<th>80 g/l</th>
<th>100 g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>cv. 851</td>
<td>0.523±0.127 a</td>
</tr>
<tr>
<td>cv. Desiree</td>
<td>0.625±0.215a</td>
</tr>
</tbody>
</table>

**Figure 2.** Tuber induction percentage under different conditions in *Solanum tuberosum* L. cv. Atlantic in vitro (Same letters indicate no differences within the group at 0.05 level).

**Figure 3.** Tubers induced under different conditions in *Solanum tuberosum* L. cv. Atlantic in vitro. A-C: 80g/l under 24-h, 16-h and 0-h photoperiod; D-F: 100g/l under 24-h, 16-h and 0-h photoperiod.

**Effect of photoperiod on tuber induction in MS with high sucrose in *Solanum tuberosum* L. cv. Atlantic**

The TIPs of 80g/l sucrose under 0, 16 and 24-h photoperiod were 70.0%, 68.3% and 78.3%, which were higher than 100g/l sucrose, respectively, but the difference was not significant except under 24-h photoperiod (Figure 2, 3). Similarly, no significant differences were in MS with same sucrose concentrations among different photoperiods. However, the germination rates of tubers induced under 16-h light were higher than those under 0-h light (Figure 4). Although the germination rates of tubers from 80g/l sucrose showed no dramatic differences with those from 100g/l sucrose under 16-h light the seedlings from the later were stronger than the former (Figure 5). Therefore, tubers induced in MS with high sucrose under 16-h photoperiod can be used for further studies.

**Effect of node on tuber induction in MS with high sucrose in *Solanum tuberosum* L. cv. Atlantic**

Cuttings with different node numbers were induced in MS with 80g/l and 100g/l sucrose under 16-h photoperiod (Figure 6). The TIPs from 0, 1 and 2 nodes were 0, 43.3% and 75.0%, respectively. In MS with 80g/l sucrose the TIP from 2 nodes was significantly higher than that from 1 node, but in MS with 100g/l sucrose there was no significant difference. Therefore, the cutting with 2 nodes was chosen to induce tuber in MS with high sucrose.

**The influences of high sucrose in MS on contents of endogenous sucrose and starch of *Solanum tuberosum* L. cv. Atlantic in vitro**

Cuttings with 2 nodes were induced in MS with 80g/l and 100g/l sucrose under 16-h photoperiod. On the 19th day the induced cuttings and corresponding tubers were harvested separately to study contents of endogenous sucrose and starch, as well as activities of sugar metabolism enzymes.
Sucrose content in induced cuttings was significantly higher than that in corresponding tubers, but starch content were opposite in both sucrose concentrations (Figure 7). With the increase of sucrose concentration in MS endogenous sucrose content dramatically increased and starch content decreased in individually cuttings and tubers. Meanwhile, endogenous sucrose content had no correlation with starch content.

**The influences of exogenous high sucrose concentration on sugar metabolic enzymes of Solanum tuberosum L. cv. Atlantic in vitro**

The activity of acid invertase in induced cuttings was higher than that in corresponding tubers, and the difference was significant in MS with 80 g/l sucrose (Figure 8). With the sucrose concentration increase in MS activity of acid invertase increased in induced cuttings and in tubers individually. Susy and SPS accumulated only in tubers, but no significant differences of Susy and SPS were between 80 g/l and 100 g/l sucrose (Figure 9).
Figure 8. Invertase activity of Solanum tuberosum L. cv. Atlantic in MS with 80g/l and 100g/l sucrose (Same letters indicate no differences within the group at 0.05 level).

Figure 9. Activities of sucrose synthase and sucrose phosphate of Solanum tuberosum L. cv. Atlantic in MS with 80g/l and 100g/l sucrose (Same letters indicate no differences within the group at 0.05 level).

DISCUSSION

Potato microtubers produced in vitro are widely used in many areas of agriculture as materials for research (Cenzano et al., 2006, 2008; Gargantini et al., 2009; Raices et al., 2003a, 2003c; Si et al., 2008). Therefore defining suitable induction factors is very important to in vitro. Nistro et al. (2010) listed high sucrose, short day photoperiod, nitrogen and temperature as the important factors to induce tubers in vitro. Among them Sucrose is considered the most critical stimulus (Ewing & Struik, 1992; Wang & Hu, 1982). Some researchers proved that increasing sugar concentrations can promote tuber formation (Raices et al., 2003b; Simko, 1994; Sonnewald et al., 1997; Xu et al., 1998). The same results were obtained in our study that tubers could be induced by high sucrose ranging from 50g/l to 100g/l and sucrose of 80 and 100g/l could induce more tubers in three cultivars of Solanum tuberosum L. in vitro. For sucrose of 50g/l and 60g/l induced fewer tubers, some researchers combined them with phytohormones to induce more tubers in vitro (Aslam et al., 2011; Ebadi et al., 2011; Hussain et al., 2006; Leclerc et al., 1994).

Potato (Solanum tuberosum L.) tubers are formed under short days, so microtubers are induced under short day or continuous darkness during culture conditions (Altindal & Karadoğan, 2010; Aslam et al., 2011; Dhital & Lim, 2012; Hoque, 2010; Hussain et al., 2006; Motallebi-Azar et al., 2013; Servet et al., 2000). Microtubers provided a valuable tool for study of genetic transformation (Gargantini et al., 2009; Si et al., 2008), which was followed by regeneration of plants from in vitro grown microtubers (Ishida et al., 1989). In our study darkness did not significantly improve TIP compared to long-day photoperiods in MS with 80g/l and 100g/l sucrose, but tubers induced under 16-h photoperiod germinated much more than under darkness. It seemed that photoperiods had no dramatic influence on TIP when exogenous sucrose concentrations ranged from 80g/l to 100g/l. The average dormancy duration of microtubers induced in continuous darkness was 210 days, but under 8-h photoperiod it was shortened to 60 days for the same cultivar (Estrada et al., 1986; Tovar et al., 1985). Microtubers produced under light had more number of eyes than those produced in the dark (Gopal & Minocha, 1998). In our experiment the reasons of higher germinations of tubers induced under 16-h photoperiod might be both. Therefore, tubers induced under 16-h photoperiod will be better to use for further studies.

Nodal section of the shoot or nodal segments/cuttings were used as explants in in vitro tuber induction (Altindal & Karadoğan, 2010; Aslam et al., 2011; Dhital & Lim, 2012; Hoque, 2010; Hussain et al., 2006; Motallebi-Azar et al., 2013; Servet et al., 2000). In our work no node no tuber meant that node was very important to tuber induction in MS medium with high sucrose. Although production of potato microtubers in vitro had been studied for a long time, people still keep on trying to look for a simple, speedy and productive induction method (Altindal & Karadoğan, 2010). Based on our study cutting with no less than 1 node induced by high sucrose could fulfil microtuber production in vitro.

Developing tubers become the largest sinks and a large proportion of sucrose taken up into this sink-tissue was converted to starch during the formation of the potato tuber (Gargantini et al., 2009; Oparka et al., 1990; Prat et al., 1990; Visser et al., 1994; Wright et al., 1989). Khuri & Moorby (1995) found that the translocation and usage of sucrose was faster than the other sugars in the tubering plantlet in vitro. Starch deposition in potato axillary buds was an indicator of microtuber induction and initiation in a sucrose-containing medium (Berljak & Pevalex-Kozliina, 1997). In our work high levels of exogenous sucrose resulted in a dramatic high gradient of endogenous sucrose content between induced cuttings and corresponding tubers, which led sucrose to flow. Such flow was beneficial to starch accumulation in tubers in MS with high sucrose. However, high sucrose content did not mean high starch content in tissues.

Sucrose can be degraded by either invertases or Susy in most plants (Koch, 2004; Kruger, 1990). SPS is the plant enzyme thought to play a major role in sucrose biosynthesis. Invertases increased concomitantly with the increase in sugar accumulation in storage tissue (Hatch...
and Glasziou, 1963). But Zhu et al. (1997) got different conclusion that invertease activity had a negative relationship with sucrose accumulation in sugarcane. Our result showed that high content of endogenous sucrose was coordinated with a high activity of invertease during in vitro tuber induction. High sucrose levels increased Susy in potatoes in culture medium (Sowokinos and Varns, 1992). High activity of Susy is found in rapidly growing tissues (Kruger, 1990) and can be used to measure sink strength (Sung et al., 1989). In our study Susy and SPS accumulated only in tubers, but no significant differences between 80g/l and 100g/l sucrose. Therefore sucrose in cuttings was hydrolyzed mainly by invertases but sucrose balance in induced tuber was maintained by Susy and SPS in MS with high level of exogenous sucrose.

In conclusion high levels of exogenous sucrose and nodal cuttings were essential to in vitro tuber induction in potatoes. In the process of induction high levels of exogenous sucrose resulted in differential distribution of sucrose, which triggered differential accumulation of starch and differential activities expression of sugar metabolizing enzymes in cuttings and in corresponding tubers.

ACKNOWLEDGMENT

This research was supported financially by China Agricultural Industry Technology System (CARS-10). We are grateful to Dr. Gülden Haspolat, of the Aeganean Agricultural Research Institute, Department of Horticulture-Ornamental Plant, for her modification and advices.

LITERATURE CITED


