Regeneration in 'Macspur' Apple by Sorbitol (D-Glucitol) and Related Carbon Sources

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Abstract. In vitro shoot proliferation and rooting of the apple scion cultivar 'Macspur' (mature culture) and of open-pollinated seedlings of 'Macspur' (seedling culture) were studied in response to the carbon sources, sorbitol, sucrose, glucose, and fructose, each supplied at 10, 30, 50, and 70 g/liter, or to the presence of varying proportions of sorbitol between 25% and 100% in conjunction with sucrose (30 g/liter total). Although the highest shoot number and shoot fresh weight of both cultures occurred with 30 g/liter sorbitol and sucrose, respectively, a medium with 25% sorbitol and 75% sucrose was best for shoot production. There was a decreasing trend in leaf/stem (fresh weight) ratio of shoots as the percentage of sorbitol increased in the medium. On rooting media supplied with 10-70 g/liter of each carbon source, rooting was best with 30 g/liter sucrose. In the mature culture (initially sucrose-grown), enhance shoot proliferation and rooting occurred on sorbitol medium after this source was subcultured 3 times on sorbitol medium.

Sorbitol (D-glucitol) has been demonstrated in vivo to be an active carbohydrate metabolite of major significance to the apple and related species of the Rosacea (2, 3, 9). In vitro, sorbitol was utilized effectively as an energy source by cells (12) and by callus of various apple genotypes and related woody species (4, 5, 13). Studies by Pua and Chong (14) on the influence of the carbon sources, sorbitol, sucrose, glucose, and fructose, during stages of in vitro propagation of the apple rootstock, Malus robusta Rehd. No. 5, demonstrated that sorbitol was required for initiation of meristem tip explants, and was the most effective carbon source for shoot multiplication; sorbitol and sucrose were equally effective for in vitro rooting. In this related study, we examined further the comparative influence of sorbitol, sucrose, glucose, and fructose on shoot proliferation and rooting of apple cultures derived from a mature fruiting tree of 'Macspur' and seedlings of 'Macspur'.

Materials and Methods

Stock cultures

The first culture, herein referred to as the mature culture, originated from 1 mm meristem tips excised from buds of a 10-year-old mature fruiting tree of 'Macspur', a spur-type mutant of Malus domestica Borkh 'McIntosh'. The 2nd culture, herein referred to as the seedling culture, originated from 5 mm shoot tips of aseptically germinated open-pollinated seedlings of 'Macspur'. In experiments described below, the mature culture was used 18 to 29 months after establishment, and the seedling culture 21 to 34 months after establishment.

Stock cultures were maintained by monthly transfer of 5 mm shoot tips to 100 x 20 mm petri dishes with 40 ml of medium or to 93 x 92 mm ointment jars with 60 ml of medium. The basal culture medium consisted of Murashige and Skoog macro- and micro-elements (11) with iron added as 0.08 mm (30 mg/liter) FeNa2EDTA (13% Fe; J.T. Baker Chemical Co.), and the following constituents, expressed in molarity (mg/liter in brackets): myo-inositol, 0.56 mm (100); thiourea, 0.33 mm (25); asparagine, 1.4 mm (180); glycine, 27 mm (2.0); thiamine HCl, 0.02 mm (0.2); and pyridoxine HCl, 0.02 mm (0.2).
In all investigations reported herein, media constituents were added and the pH adjusted to 5.7 before autoclaving at 1 kg/cm² and 121°C for 15 min, except for fructose, which was filter-sterilized (4). Cultures were maintained at 26° ± 2° under 16-hr daily photon flux density of 51.5 μmol s⁻¹ m⁻² from cool-white fluorescent lamps.

Shoot proliferation. Shoot tips of stock cultures were transferred to media described previously supplied with a series of concentrations of each of the following carbon sources (expressed in mM): sorbitol, 54.9, 164.7, 274.5, 384.3; sucrose, 29.2, 87.6, 146.1, 204.5; and glucose or fructose, 55.5, 166.5, 277.5, 388.6. Each of these series of concentrations was equivalent to 10, 30, 50, and 70 mg/liter of carbon source. Another series of investigations compared the response of stock cultures maintained continuously on 30 g/liter sucrose medium (C-SUC) and stock cultures of similar origin grown on 30 g/liter sorbitol medium through 3 subcultures (3-SOR). Shoot tips (5 mm) from these cultures were transferred to media supplied with sorbitol and sucrose combinations (30 g/liter total) with varying proportions of each, i.e., sorbitol (%) + sucrose (%): 0 + 100, 25 + 75, 50 + 50, 75 + 25, and 100 + 0. In these experiments, number of shoots and shoot fresh weight were evaluated after 4 weeks. Fresh weight of leaf and stem, leaf/stem (fresh weight) ratio, and/or compactness index (CI) of cultures also were evaluated to investigate differential growth and morphology due to carbon source treatments. CI was evaluated as:

\[
CI = \frac{H \times W_1 \times W_2}{N} \times 1000
\]

where H is the height (mm), W₁ and W₂ are the narrowest and widest width (mm), respectively, and N is the number of shoots (14). There were 4 replications and 5 shoot explants per treatment in a 125-ml Erlenmeyer flask containing 30 ml of medium. Each experiment was repeated twice, the results were pooled, and data were analyzed statistically as a factorial experiment.

Rooting. Shoot cuttings (2–3 cm) of stock cultures were implanted on half-strength (mineral salts) agar medium with 50 mg/liter inositol and amounts of IBA which in previous investigations yielded 100% rooting, i.e., 1.25 μM (0.25 mg/liter) for the seedling culture, and 15 μM (3.0 mg/liter) for the mature culture. In these investigations, IBA was tested at 8 concentrations between 0 and 35 μM (0 and 7.0 mg/liter). The influence of a series of concentrations of each of the 4 carbon sources, and also of sorbitol and sucrose combinations, was evaluated in the rooting media as described previously for shoot proliferation media. Each experiment consisted of 4 replications and 5 cuttings per treatment in 125-ml Erlenmeyer flasks containing 40 ml of medium. Rooting response was expressed in terms of percent rooting. Data were transformed to arcsin √N before analysis of variance as previously described.

Results

Shoot proliferation. When seedlings of 'Macspur' and mature 'Macspur' cultures, previously initiated and maintained on 30 g/liter sucrose medium, were transferred to experimental proliferation media supplied with 10, 30, 50, and 70 g/liter each of sorbitol, sucrose, glucose, and fructose (Fig. 1), the highest number of shoots of both cultures (seedling, 10.1; mature, 3.9) occurred with 30 g/liter sorbitol. The highest shoot fresh weights (seedling, 469 g; mature, 415 g) occurred with 30 g/liter sucrose (Fig. 2). Although shoot leaf/stem ratio of both cultures tended to decrease with increasing carbon source concentrations, the lowest ratios occurred on sorbitol and(or) fructose media (Table 1).

The addition of 25% (weight basis) of sorbitol to the medium increased shoot number and weight of shoots, leaves, and stem over all-sucrose medium for both cultures (Table 2). More sorbitol in the medium showed inconsistent or no further increase in shoot number or shoot weight but resulted in a decreasing trend in leaf/stem ratio (Table 2). This decrease was attributed to decreasing leaf weight and increasing stem weight as the
Fig. 2. Influence of carbon sources and concentrations on shoot fresh weight of seedlings of 'Macspur' and mature 'Macspur' cultures. Vertical bars represent LSD at 5% level.

Discussion

Different apple genotypes are known to respond differently to the same medium and environmental conditions during establishment, proliferation, and rooting in vitro (20, 21). In comparison with the mature 'Macspur' culture, the culture of 'Macspur' seedlings was more prolific and more compact in appearance, with relatively short internodes, small leaves, and large stems (lower leaf/stem ratio, Table 1); leaves were more serrated and darker green in color; the IBA requirement for rooting was lower, and rooting was quicker. Whereas these differences can be related to differences in genotype and growth phase of the 2 cultures (1, 10, 17, 20), there were other morphological and physiological differences both within and between cultures due to type and/or composition of the C source fraction. Although the highest shoot number and shoot fresh weight of both cultures occurred on sorbitol and sucrose media, respectively, a medium with 25% sorbitol and 75% sucrose was best for shoot production of these cultures. This medium provided a relatively large increase in number of less compact shoots that, from a practical point of view, were easier to handle during transfers.

Studies have shown that a C source in the nutrient medium is essential for in vitro rooting of apples (19). In the present study, sucrose was best for rooting. Interestingly, the seedling culture (sucrose-grown) rooted better (50% to 95%) than the mature culture (10% to 40%) on sorbitol medium (Fig. 3) but showed a large reduction in rooting (5% to 55%) in relation to the mature culture (80% to 100%) after both were subcultured 3 times with sorbitol (Table 3). This 'sorbitol effect' indicates marked changes in the carbohydrate metabolism of the cultures and appears to be related primarily to degree of adaptation to the medium (19, 20).

Since sorbitol and related sugars are normally readily interconvertible (2), these changes may be associated with a lack of or unavailability of the enzymes associated with catalysis of sorbitol (14). According to Thorpe (16), initiation of organized development involves a shift in metabolism in which new enzymes originally absent are synthesized, or enzymes that are present show increased synthesis. The enhanced shoot production of the 3-SOR mature culture described previously, and increased growth of sucrose-grown *Prunus persica* callus upon subculture with sorbitol (5), appear to be similar occurrences. It would be interesting to observe the response of apple cultures initiated on sorbitol medium and later subcultured on sucrose medium.

Previously with 'Macspur', Lane and McDougald (8) obtained 4.6 shoots per culture after 40 days, somewhat similar to the 3.9 shoots we obtained after 28 days (Fig. 1). In another study, Lane et al. (1) obtained about 16 shoots per culture after...
Table 1. Influence of C sources and concentrations on leaf/stem ratio of seedlings of ‘Macspur’ and mature ‘Macspur’ cultures.

<table>
<thead>
<tr>
<th>C source</th>
<th>Conc (g/liter)</th>
<th>Seedling culture</th>
<th>Mature culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>0.74z</td>
<td>0.29</td>
<td>0.24</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1.32</td>
<td>0.61</td>
<td>0.29</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.91</td>
<td>0.51</td>
<td>0.51</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.69</td>
<td>0.26</td>
<td>0.33</td>
</tr>
<tr>
<td>Mean</td>
<td>0.91</td>
<td>0.42</td>
<td>0.34</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>C source, 0.09; Conc 0.09; Interaction, 0.18</td>
<td>C source, 0.12; Conc 0.12; Interaction, 0.23</td>
<td></td>
</tr>
</tbody>
</table>

z Each datum represents the mean of 4 replications each with 5 shoots recorded after 4 weeks in vitro.

Table 2. Shoot proliferation of seedlings of ‘Macspur’ and mature ‘Macspur’ cultures in response to sorbitol and sucrose treatment combinations (30 g/liter total concentration).

<table>
<thead>
<tr>
<th>C source</th>
<th>No. of shoots</th>
<th>Shoot fresh wt (mg)</th>
<th>Leaf fresh wt (mg)</th>
<th>Stem fresh wt (mg)</th>
<th>Leaf/stem ratio</th>
<th>Cl(^y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorbitol</td>
<td>+ Sucreose</td>
<td>Seedling Mature</td>
<td>Seedling Mature</td>
<td>Seedling Mature</td>
<td>Seedling Mature</td>
<td>Seedling Mature</td>
</tr>
<tr>
<td>0</td>
<td>+ 100</td>
<td>8.1z</td>
<td>3.2</td>
<td>351</td>
<td>335</td>
<td>122</td>
</tr>
<tr>
<td>24</td>
<td>+ 75</td>
<td>12.9</td>
<td>5.9</td>
<td>417</td>
<td>367</td>
<td>144</td>
</tr>
<tr>
<td>50</td>
<td>+ 50</td>
<td>11.8</td>
<td>7.1</td>
<td>383</td>
<td>384</td>
<td>112</td>
</tr>
<tr>
<td>75</td>
<td>+ 25</td>
<td>12.3</td>
<td>6.3</td>
<td>382</td>
<td>397</td>
<td>101</td>
</tr>
<tr>
<td>100</td>
<td>+ 0</td>
<td>13.4</td>
<td>6.6</td>
<td>370</td>
<td>358</td>
<td>81</td>
</tr>
<tr>
<td>LSD 5%</td>
<td></td>
<td>1.9</td>
<td>2.4</td>
<td>21</td>
<td>14</td>
<td>8</td>
</tr>
</tbody>
</table>

z Data were analyzed separately for the seedling and the mature culture. Within columns, data are main effects of C source (sorbitol + sucrose treatment combinations, factor B) averaged over two culture sources (factor A); there was no A X B interaction.

y Cl = compactness index.

42 days. In rooting experiments with ‘Macspur’, using naphthaleneacetic acid (NAA) between 0.1 and 33 \( \mu \text{g} \) (0.02-6.1 mg/liter), Lane and McDougald (8) obtained the highest rooting of 58% with 1.0 \( \mu \text{g} \) (0.2 mg/liter) NAA. We obtained 100% rooting of the mature ‘Macspur’ culture with 15 \( \mu \text{g} \) (3 mg/liter) IBA. Zimmerman (private communication) reported similarly high rooting of the related cultivars, ‘McIntosh’ and ‘Paladino Spur McIntosh’ using a much lower IBA concentration of 1.5 \( \mu \text{g} \) (0.1-0.3 mg/liter). Although low auxin concentrations have been found to be more effective than higher concentrations for rooting many other apple cultivars (18), Jones et al. (6) used IBA as high as 15 \( \mu \text{g} \) for rooting 5 apple scion cultivars, and previously we found that the difficult-to-root Ottawa 3 rootstock required even higher concentration of IBA (33.6 \( \mu \text{g} \)) for maximum rooting (15). These differences are probably related to differences in media, culture conditions, and status of the cultures (8, 20, 21).

Sucrose has been the standard C source used in nutrient media for in vitro propagation of higher plants, including numerous apple rootstock and cultivars (6, 7, 19, 21). The present study shows that sucrose was a most effective C source for culture of the 2 apple sources and demonstrated also that under certain circumstances sorbitol is beneficial as an adjunct or alternate C source.

**Literature Cited**

8. Lane, W.D. and J.M. McDougald, 1982. Shoot tissue culture of


