Abstract. Shoot tips of apples (*Malus domestica* Borkh. cv. Golden Delicious) cultured in a basal medium plus 5 mg/liter 6-benzylamino purine (BA) survived 12 months storage at 1°C or 4°C and proliferated new shoots when returned to 26°C.

Present germplasm storage of apple involves a considerable investment of land and labor to maintain trees in permanent orchard locations. In addition, the tree is exposed to the possibility of injury from various hazards including low winter temperatures, pests, and diseases. Mullin and Schlegel (2) have demonstrated that *in vitro* grown strawberry plantlets remained viable after storage at 4°C for up to 6 years. This study was conducted to investigate the feasibility of low temperature storage of apple shoots *in vitro*.

'Golden Delicious' shoot tips were proliferated in a basal medium consisting of Murashige and Skoog salts, thiamine-HCl, 0.1 mg/liter; pyridoxine-HCl, 0.5 mg/liter; nicotinic acid, 0.5 mg/liter; glycine, 2.0 mg/liter; BA, 5 mg/liter; sucrose, 30 g/liter; and agar, 10 g/liter. Proliferated shoots were explanted, 1 shoot per 25 x 150 mm glass culture tube, to fresh media and tubes were closed with plastic caps (Kaputs) and sealed with parafilm to prevent excessive drying. Forty tubes were placed in the dark at each of the following temperatures: -17°C, 1°C, and 4°C, with 40 tubes held under normal conditions (26°C, 16 hr at 1500 lux) as controls. Every 3 months, 10 tubes were removed from each storage treatment and placed at 26°C with 16 hr of light at 1500 lux to determine proliferation potential after storage. The number of shoots (explants) that developed were estimated when the tubes were removed from each temperature treatment after 3, 6, 9, or 12 months storage (Fig. 1), and dissected and counted after an additional 1 month of growing at 26°C. At the same time the number of shoots surviving and the proliferation rate of surviving shoots was determined for the controls.

Shoots stored at -17°C did not survive the thawing process. Shoots stored at 1°C or 4°C showed no decrease in survival for up to 6 months but 2 tubes at 1°C were contaminated at 9 months and 1 tube at 4°C showed contamination at the 12 months sampling date. Shoots maintained at 26°C had high losses due to a combination of contamination, desiccation and nutrient depletion, and by 12 months shoots in only 1 culture tube of the original 40 remained alive.

There was a 3 to 7-fold proliferation after 1 month when shoots stored at 1°C and 4°C were moved to 26°C. This was in the range of proliferation observed for apple shoots maintained in fresh media but not stored (1). However after 12 months of cold storage, the rate of proliferation increased. Bud development was apparent at the end of the 12 month cold storage treatment. Apparently bud development occurs at these low temperatures, albeit very slowly.

The proliferation of the single surviving shoot stored at 26°C to 14 shoots was the accumulated proliferation over 13 months. In this treatment there were originally 40 shoots (1 shoot each of 40 tubes) which reduced to 14 shoots (1 tube with 14 potential explants).

Table 1. Effect of storage temperatures on survival and proliferation of apple shoots *in vitro*.

<table>
<thead>
<tr>
<th>Time in storage (mo)</th>
<th>-17°C</th>
<th>1°C</th>
<th>4°C</th>
<th>26°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>After storage</td>
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<tr>
<td>+ 1 month at 26°C</td>
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<td></td>
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<tr>
<td>After storage</td>
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<td>+ 1 month at 26°C</td>
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<td>After storage</td>
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<td>After storage</td>
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<tr>
<td>+ 1 month at 26°C</td>
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</tr>
<tr>
<td>Survival (%)</td>
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<tr>
<td>3</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>80</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>90</td>
</tr>
</tbody>
</table>

### Literature Cited


### Table 1. Effect of storage temperatures on survival and proliferation of apple shoots *in vitro*.

<table>
<thead>
<tr>
<th>Storage temperature</th>
<th>1°C</th>
<th>4°C</th>
<th>26°C</th>
</tr>
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<tbody>
<tr>
<td>Survival (%)</td>
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</tr>
<tr>
<td>Avg no. of shoots per culture tube</td>
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<tr>
<td>3</td>
<td>1.1a</td>
<td>1.4b</td>
<td>1.2c</td>
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<tr>
<td>6</td>
<td>1.6c</td>
<td>6.2b</td>
<td>2.1c</td>
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<tr>
<td>9</td>
<td>1.5c</td>
<td>6.9b</td>
<td>2.1c</td>
</tr>
<tr>
<td>12</td>
<td>1.3c</td>
<td>11.0a</td>
<td>2.2c</td>
</tr>
</tbody>
</table>

*Mean separation within columns is by Duncan's multiple range test, 5% level.*

**Fig. 1.** Apple shoot after 9 months storage at 1°C.

Our results indicate that apple shoots can be stored at 1°C or 4°C for at least 1 year with no loss of growth potential. The survival of cultured apple shoots at low temperature storage indicates an alternative to orchard culture for germplasm preservation. About 2 thousand culture tubes could be stored in an ordinary 0.28 m³ (10 ft³) refrigerator. It would require 5.7ha (14 acres) to accommodate the same number of trees at spacing of 4.6 x 6.1 m (12 x 20 feet).

Our results suggest that *in vitro* culture would accommodate indefinite storage of germplasm and could also facilitate germplasm shipments. This opens up the technological feasibility of a world repository of apple germplasm.