Increasing In Vitro Shoot Elongation and Proliferation of ‘G.30’ and ‘G.41’ Apple by Chilling Explants and Plant Growth Regulators

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Abstract. These studies were conducted to determine the most effective methods for increasing shoot elongation during the initial proliferation stage of micropropagation in two dwarfing apple, *Malus × domestica* (Borkh.), rootstock cultivars. Several experiments were conducted to compare explant collection date, exposure to chilling (5 ± 1 °C) temperatures, and varying concentrations of plant growth regulators in Murashige and Skoog (MS) media. Microshoot growth of ‘Geneva 41’ (‘G.41’) was very low and unafflicted by chilling duration from 0 to 8 weeks or by gibberellic acid (GA3) concentration from 0 to 1.0 mg·L⁻¹, but was improved by an additional subculture which increased shoot length from 1 to 15 mm. In ‘Geneva 30’ (‘G.30’), shoot elongation was most affected by date, chilling explants, and by optimizing cytokinin concentration and type. Explant collection date in April increased shoot growth compared with August or November. Microshoot growth of ‘G.30’ was increased by chilling nodal explants for 4 and 6 weeks when explants were collected in August and November, but not in April. Eight weeks chilling was detrimental for explants collected in April, and generally had little or no effect with August and November. The cytokinin 6-benzylaminopurine (BA) increased shoot number to a greater extent than thidiazuron (TDZ) or zeatin (ZT), and was also more effective for increasing shoot elongation with concentrations of 0 to 2.0 mg·L⁻¹. In ‘G.30’, GA3 increased shoot growth at the optimum concentration of BA, but not with lower concentrations. ‘G.30’ microshoots were fewer and shorter with 24-epibrassinolide (EBR) at concentrations of 0.1 and 1.0 mg·L⁻¹. Chemical names: N-phenyl-N-(1,2,3-thiadiazol-5-yl)urea (TDZ), 6-(4-hydroxy-3-methylbut-2-enylamino)purine (ZT).

Micropropagation can rapidly increase numbers of stock plants, particularly for new cultivars that are available in limited quantities. Because of their genetic stability and potential for high proliferation, single- and multiple-node shoot pieces are commonly used as explants for in vitro propagation of apple (De Klerk, 1992). Efficient propagation using this method depends on rapid shoot development and elongation following establishment. However, many important apple rootstock cultivars exhibit a lack of shoot elongation during the proliferation phase with the foliage appearing as a rosette (Aklan et al., 1993; van Nieuwkerk et al., 1986). Thidiazuron (TDZ) causes greater proliferation than BA, but can also inhibit elongation to a greater extent (Huetteman and Preece, 1993; Marin et al., 1993; van Nieuwkerk et al., 1986). Compared with BA, kinetin results in fewer but longer shoots (Lundegan and Janick, 1980) and may be more effective for genotypes that display poor shoot elongation.

Adding auxin or GA3 to the media can increase shoot length (Kane, 2003; Reeves et al., 1985), but the effect is variable and dependent on a number of other factors such as the concentration of cytokinin in the media. Pua et al. (1983) reported that low concentration of BA combined with GA3 increases shoot number and length, but GA3 decreases shoot length with a high BA concentration in the media. Increasing concentration of indole-3-butyric acid (IBA) increases shoot length, but only when GA3 is...
also present (Yepes and Aldwinckle, 1994). A greater understanding of the GA₃ effectiveness and interactions with other growth regulators may improve propagation of recalcitrant genotypes.

Brassinosteroid promotes in vitro shoot multiplication and elongation in apple when applied directly to foliage (Schafer et al., 2002), but this method is labor intensive and increases the chance for contamination. When added to media, EBR can promote shoot elongation at a concentration below 1 mg·L⁻¹ (Schaefer et al., 2002). As an additive to media, it has not been tested for increasing in vitro shoot growth in apple cultivars that display shoot growth inhibition.

The purpose of these studies was to fill substantial gaps in our understanding of the effects of explant collection date, chilling nodal explants, and media concentration of the plant growth regulators GA₃, EBR, BA, ZT, and TDZ on shoot growth of ‘G.30’ and ‘G.41’ apple during the initial proliferation stage of micropropagation. The focus of this research was to identify factors that improve initial shoot proliferation. Therefore, the effect of these factors on continued proliferation and subsequent rooting was not examined.

Materials and Methods

Plant materials. One- and two-year-old ‘G.30’ and ‘G.41’ apple trees, that served as stock plants, were overwintered in cold storage in the dark at 1 ± 1 °C as potted plants from Oct. 2010 to Apr. 2011 and again from Oct. 2011 to Mar. 2012. Trees were then grown in a heated greenhouse at the University of Maine (Orono, ME) to serve as stock plants from Oct. 2010 to Apr. 2011 and again from Mar. 2012. Trees were then aged in the dark at 1 ± 1 °C and then transferred into Magenta® square vessels containing distilled water and transferred to a tissue culture laboratory. To reduce transthy, collected shoots were defoliated and trimmed to 10- to 15-mm length with one or two nodes. The nodal segments were firstly surface-disinfested for 30 s in 70% alcohol and then soaked for 10 min in an aqueous solution of 10% bleach (6.0% sodium hypochlorite; The Clorox Company, Oakland, CA) and several drops of Tween 20 (Agdia® Inc., Elkhart, IN). All nodal cuttings were rinsed three to five times under a laminar hood using sterilized, distilled water.

Murashige and Skoog (1962) basic media supplemented with 30 g·L⁻¹ sucrose and 8 g·L⁻¹ agar (Sigma Chemical Co., St. Louis, MO) was used in all experiments. The pH of all media was adjusted to 5.8 ± 0.2 with 1 M NaOH or 1 M HCl solution after adding agar. An aliquot of 10 mL of the medium was pipetted into glass tubes, capped and autoclaved at 121 °C for 30 min. Establishment media contained no plant growth regulators. For experimental proliferation media, cytokinins or IBA were added to media before autoclaving, but GA₃ and EBR were added after a period to allow cooling to a temperature of 60 ± 10 °C. To prevent contamination by the nonautoclaved GA₃ and EBR, they were first dissolved in 95% ethanol. Each nodal cutting was dissected and vertically inserted into 60-mL disposable culture tubes (borosilicate glass, 25 × 150 mm) containing 10 mL previously prepared MS culture media for establishment. Tubes with explants were sets in racks and sealed in a plastic bag. Phenolic browning was minimized by growing stock plants in a greenhouse without supplemental lighting and by selecting the youngest portion of new shoots. Before and after chilling treatment, cultures were arranged randomly on shelves under cool-white fluorescent lamps (Philips 60 W 4 ft T8) and incubated at a temperature of 27.2 ± 1.9 °C with a 16-h photoperiod. Photosynthetic photon flux was 25 μmol·m⁻²·s⁻¹ as measured using a quantum light meter (LQM 70-10; Apogee Instruments Inc., Logan, UT). The relative humidity in the culture room was 70 ± 5%. In each experiment, shoots were cultured under the same conditions as the establishment phase.

Experimental treatments. To compare explant collection dates and chilling duration, ‘G.30’ explants were collected on three different dates: 1) Aug. 2011 from trees placed in greenhouse in Apr. 2011, 2) Nov. 2011 from the same stock plants, and 3) Apr. 2012 from a different set of trees that were placed in the greenhouse in Mar. 2012. Trees had been overwintered in a cold storage room at 1 ± 1 °C from October to March in both years. Stock plants of ‘G.41’ failed to produce a sufficient number of shoots for this experiment. Nodal explants were cultured on establishment media in an incubator at a temperature of 4 to 6 °C for 0, 4, 6, or 8 weeks before moving into the culture room for the establishment phase. After 40 d of establishment culture, the newly sprouted shoots were cut off and transferred into Magenta® square vessels containing 50 mL fresh MS media supplemented with 1.0 mg·L⁻¹ IBA and 0.5 mg·L⁻¹ BA, and 0.1 IBA, and 1.0 mg·L⁻¹ IBA. Media also contained different concentrations of GA₃ which were 0, 0.5, or 1.0 mg·L⁻¹.

To compare GA₃ concentration in the first and second subculture, ‘G.41’ explants were collected in Apr. 2013 from trees placed in greenhouse in Mar. 2013. Explants were aseptically established and prepared as in previous experiments and were transferred into GA-7 vessels containing 50 mL fresh MS media with 2.0 mg·L⁻¹ BA, 0.5 mg·L⁻¹ IBA, and 0.1 or 1.0 mg·L⁻¹ of EBR.

To compare different chilling durations and GA₃ concentrations, explants of ‘G.41’ were collected in Apr. 2013 from trees placed in the greenhouse in Mar. 2013. Nodal explants were cultured on establishment media in an incubator at a temperature of 5 ± 1 °C for 0, 4, 6, or 8 weeks before moving into the culture room for the establishment phase.

Shoot growth measurements. After culturing for 40 d on proliferation media, the length of the longest microshoot per explant, and the number of elongated microshoots were measured in each experiment. Elongation was defined as a minimum shoot length of 15 mm. Shoots were characterized as elongated with a score of 1 or nonelongated with a score of 0. For each treatment
combination, the multiplication rate was calculated as the percentage of explants with two or more shoots and elongation rate as the percentage of microshoots with a shoot length of 15 mm or longer.

Data analysis. In all experiments, we used a completely randomized design with at least four replicates per treatment and eight explants per replicate. All data were analyzed using Statistical Analysis Systems (Version 9.3; SAS Institute Inc., Cary, NC). Analysis of variance was used to test for variation in the maximum in vitro shoot length. The multiplication and elongation rates were analyzed as binomial data, and the number of microshoots per explant was analyzed as negative binomial data. Means separation was carried out using Fisher’s least significant difference method with an alpha of 0.05.

Results

Chilling duration and explant collection date affected the number of ‘G.30’ shoots proliferated per explant, multiplication rate, shoot length, and the number of elongated shoots (Table 1). In unchilled explants, collection in April resulted in a greater number of shoots and a greater multiplication rate compared with August and November. Chilling explants collected in April did not increase the number of shoots or multiplication rate, but chilling for 8 weeks decreased both compared with no chilling. Chilling explants collected in August and November for 6 weeks increased shoot number, but other durations had no effect, whereas all three chilling durations increased the multiplication rate. Shoot elongation was generally greater with explants collected in April compared with August and November. In April, chilling for 4 weeks had no effect on shoot length or elongation rate, 6 weeks increased shoot length, but not the elongation rate, and 8 weeks decreased both. In August and November, 6 weeks of chilling increased shoot length and elongation rates, whereas 4 weeks increased only the elongation rate in August and 8 weeks had no effect on either. Chilling explants collected in August and November increased proliferation to the level that occurred with the April collection.

Type of cytokinin influenced ‘G.30’ shoot number, multiplication rate, shoot length, and elongation rate with an interaction with concentration (Table 2). The main effect of concentration was significant for shoot number, multiplication rate, and elongation rate, but not shoot length. The number of new shoots per explant and the multiplication rate were the poorest with no cytokinin in the media. For BA, there was a strong effect of concentration with 0.5 mg L\(^{-1}\) having no effect on shoot number, 1.0 mg L\(^{-1}\) slightly increasing it, and 2.0 mg L\(^{-1}\) resulting in the largest number of shoots compared with lower concentrations of BA and all rates of TDZ and ZT. Benzyladenine at 2.0 mg L\(^{-1}\) resulted in a greater multiplication rate and shoot length than lower concentrations, but the elongation rate was similar to the rate that occurred with 1 mg L\(^{-1}\) of BA. However, BA at 2.0 mg L\(^{-1}\) resulted in a multiplication rate similar to TDZ. For TDZ, there was no concentration effect on shoot growth at the rates tested in this experiment. The number of shoots per explant and the multiplication rates were generally good with TDZ, but shoot length was the shortest and none of the shoots grew in length more than 15 mm. ZT increased shoot number at the lowest concentration, but higher concentrations had no effect compared with no cytokinin. Multiplication rate was also increased by ZT at the two lower rates compared with no cytokinin. With 0.5 and 1.0 mg L\(^{-1}\) ZT, shoot length was similar to 0.5 mg L\(^{-1}\) BA, but was shorter than BA at 1.0 to 2.0 mg L\(^{-1}\). Shoot length with the highest rate of ZT was similar to no cytokinin. At equivalent concentrations, the elongation rate with ZT was less than with BA, but at 0.5 and 1.0 mg L\(^{-1}\) was greater than equivalent concentrations of TDZ.

Table 1. In vitro shoot growth of ‘G.30’ apple from three explant collection dates and following chilling

<table>
<thead>
<tr>
<th>Date</th>
<th>Chilling (wk.)</th>
<th>No. of shoots/explant</th>
<th>Multiplication rate (%)</th>
<th>Shoot length (mm)</th>
<th>Elongation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apr. 2012</td>
<td>0</td>
<td>3.0 ab*</td>
<td>78 ab</td>
<td>21 bc</td>
<td>78 ab</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.4 bc</td>
<td>86 a</td>
<td>22 ab</td>
<td>71 abc</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3.4 c</td>
<td>83 a</td>
<td>26 a</td>
<td>92 a</td>
</tr>
<tr>
<td>Aug. 2011</td>
<td>8</td>
<td>1.5 d</td>
<td>44 cd</td>
<td>16 de</td>
<td>62 bcd</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.1 bc</td>
<td>73 ab</td>
<td>18 cde</td>
<td>73 abc</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2.7 b</td>
<td>73 ab</td>
<td>21 bc</td>
<td>76 abc</td>
</tr>
<tr>
<td>Nov. 2011</td>
<td>8</td>
<td>1.9 c</td>
<td>59 bc</td>
<td>16 de</td>
<td>52 cd</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.0 bc</td>
<td>54 bc</td>
<td>13 e</td>
<td>27 c</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2.9 ab</td>
<td>73 ab</td>
<td>19 ed</td>
<td>78 ab</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.9 c</td>
<td>61 bc</td>
<td>16 de</td>
<td>61 bcd</td>
</tr>
</tbody>
</table>

Table 2. In vitro shoot growth of ‘G.30’ apple on media with three different cytokinins.

<table>
<thead>
<tr>
<th>Cytokinin type</th>
<th>Conc. (mg L(^{-1}))</th>
<th>No. of shoots/explant</th>
<th>Multiplication rate (%)</th>
<th>Shoot length (mm)</th>
<th>Elongation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BA</td>
<td>0.5</td>
<td>2.4 de</td>
<td>68 b</td>
<td>14 c</td>
<td>50 b</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>3.8 d</td>
<td>74 b</td>
<td>21 b</td>
<td>79 a</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>10.6 a</td>
<td>100 a</td>
<td>25 a</td>
<td>90 a</td>
</tr>
<tr>
<td>TDZ</td>
<td>0.5</td>
<td>7.2 b</td>
<td>100 a</td>
<td>3 e</td>
<td>0 d</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>8.3 b</td>
<td>100 a</td>
<td>3 e</td>
<td>0 d</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>7.3 b</td>
<td>100 a</td>
<td>3 e</td>
<td>0 d</td>
</tr>
<tr>
<td>ZT</td>
<td>0.5</td>
<td>5.4 c</td>
<td>100 a</td>
<td>14 c</td>
<td>26 c</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>2.3 e</td>
<td>78 b</td>
<td>16 c</td>
<td>50 b</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>1.5 e</td>
<td>27 c</td>
<td>9 d</td>
<td>0 d</td>
</tr>
</tbody>
</table>

*Explants were collected in April 2012 1 month after budbreak; in August and November 2011, 4 and 7 months after budbreak, respectively.
*Percentage of explants with two or more new shoots.
*Percentage of shoots with a shoot length equal to or longer than 15 mm.
*Means followed by the same letter in each column are not significantly different by Fishers least significant difference test at α = 0.05 level.
*NS, **, *** = ns nonsignificant or significant at P ≤ 0.05, 0.01, or 0.001, respectively.
almost no effect on ‘G.30’ explants collected in late June (data not shown).

EBR negatively affected the shoot growth of ‘G.30’ (Table 4). Shoot growth significantly decreased with both concentrations of EBR, and the higher concentration decreased shoot growth more than the lower concentration.

Shoot growth of ‘G.41’ was poor (Table 5) compared with ‘G.30’, and chilling and GA3 had very little impact on its shoot growth. There was an interaction between the two factors in their effect on the number of shoots per explant and the multiplication rate. Chilling for 4 weeks slightly increased the number of shoots and the multiplication rate, but only with no GA3 added to the media. Concentrations of GA3 at 0.5 and 1.0 mg L⁻¹ had no effect on shoot number or multiplication with any duration of chilling. Shoot length and the number of elongated shoots were unaffected by chilling duration up to 8 weeks or by GA3 concentration up to 1.0 mg L⁻¹. Collection date was not tested on ‘G.41’, and all ‘G.41’ explants were collected in April.

When concentration of GA3 was tested on ‘G.41’, it had no effect on shoot growth during the first or second subculture (Table 6). Shoot multiplication and elongation were low during the first subculture, but were increased by an additional subculture on proliferation media. Shoots ‘G.41’, it had no effect on shoot growth during July. Other times were not tested for ‘G.41’ since stock plants grown in the greenhouse were insufficient new growth for additional explants. After the initial flush of shoot elongation, they formed a terminal bud and failed to elongate, and when shoot tips were removed, they failed to branch.

The condition of stock plants appeared to be one of the most important factors controlling ‘G.30’ micropropagation efficiency in this study. Although it was tested in two different years, shoot multiplication and elongation of ‘G.30’ were the greatest when explants were collected in Apr. 2012 compared with Aug. and Nov. 2011. In 2012, explants collected in July for a subsequent experiment had similar multiplication and elongation rates as April (Tables 1 and 2). In 2013, explants collected at different times also displayed a seasonal variation in shoot proliferation and elongation that varied somewhat from the previous year. A high degree of variation occurred in the number of new shoots per explant with 3.8 in April, 10.6 in May, 6.6 in early June, and 1.8 in late June

### Discussion

Commercial application of apple micropropagation is limited by inefficient multiplication rates which range from four to six new shoots per explant in a regular 1-month subculture for most cultivars (Druart, 2003). Shoot proliferation of ‘G.41’ was poor and was not improved by the addition of GA3 or chilling. Additional time in culture that occurs with subculturing can increase propagation efficiency (Kane, 2005; Webster and Geng, 2014).
Table 6. In vitro shoot growth of ‘G.41’ apple during first and second subculture on media with different concentrations of gibberellic acid (GA₃).

<table>
<thead>
<tr>
<th>Main effect</th>
<th>No. of shoots/explant</th>
<th>Multiplication rate (%)</th>
<th>Shoot length (mm)</th>
<th>Elongation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subculture</td>
<td>First 1.5 b</td>
<td>40 b</td>
<td>1 b</td>
<td>0 b</td>
</tr>
<tr>
<td></td>
<td>Second 4.2 a</td>
<td>100 a</td>
<td>12 a</td>
<td>30 a</td>
</tr>
<tr>
<td>GA₃ (mg·L⁻¹)</td>
<td>0.0 2.8</td>
<td>73</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>0.5 2.9</td>
<td>81</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>1.0 3.5</td>
<td>70</td>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td>Significance</td>
<td>Subculture ***</td>
<td>***</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>GA₃ NS NS NS NS</td>
<td>GA₃ × GA₃ NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Percentage of explants with two or more new shoots.

**Percentage of shoots with a shoot length equal to or longer than 15 mm.

***Means followed by the same letter in each column are not significantly different by Fisher’s least significant difference test at α = 0.05 level.

Despite the use of the same stock plants, culture room conditions, and media constituents. This seasonal reduction in summer in the ability to proliferate may indicate that axillary buds had acquired a level of dormancy by those collection dates (Fuchigami and Nee, 1987). Temperatures above 20 °C induce terminal bud set and dormancy in apple buds (Hauagge and Cummins, 1991a, 1991b). In our study, stock plants were grown in a greenhouse where temperatures were at times above 30 °C, which may have contributed to the poor proliferation in summer and fall.

Cultivar ‘G.30’ exhibited complex responses to chilling treatments. Six weeks of chilling in spring did not increase shoot multiplication of ‘G.30’, but chilling in summer or fall increased proliferation to the same level as occurred with April. Shoot elongation was increased by chilling explants for 6 weeks regardless of when they were collected, but explants collected in August and November were slightly shorter compared with April explantation. Most apple cultivars have a minimum chilling requirement of about 1000 h (6 weeks) or more for terminal budbreak, but additional budbreak occurs with additional chilling (Hauagge and Cummins, 1991a; Heide and Prestrud, 2005). This did not occur with ‘G.30’—since 8 weeks of chilling inhibited shoot growth compared with shorter durations. Rather than displaying signs of full dormancy that involve leaf abscission, the shoot tips continued to form and unfold new leaves without shoot elongation, giving the shoots a rosette appearance similar to what has been described in greenhouse grown apple shoots that sprout in November before any chilling temperatures (Hauagge and Cummins, 1991a). Chilling for at least 6 weeks was the most effective duration for ‘G.30’ for promoting shoot elongation, but additional chilling time appeared to be detrimental for unknown reasons.

Table 6. In vitro shoot growth of ‘G.41’ apple during first and second subculture on media with different concentrations of gibberellic acid (GA₃).

The addition of phytohormones to media resulted in proliferation and growth similar to those reported for other apple cultivars with some notable exceptions. The addition of cytokinin is essential for adequate proliferation of apple shoots (Elliott, 1972) and is typically added to proliferation media (Kane, 2005). For ‘G.30’, all three cytokinins tested were effective for increasing shoot number, but BA was more effective at the concentrations tested and also increased shoot elongation to a greater extent than ZT or TDZ. As in previous studies (Hutchinson, 1984; van Nieuwkerk et al., 1986), TDZ inhibited elongation but concentrations in our study may have been supraoptimal (Huetteman and Preece, 1993). As reported for ‘Golden Delicious’ by Lundergan and Janick (1980), ZT increased shoot growth of ‘G.30’, but only at concentrations of 1.0 mg·L⁻¹ or lower.

The optimal BA concentration varies with cultivar, but is generally 1.0 to 2.0 mg·L⁻¹ for apple (Hutchinson, 1984; James and Thurbon, 1981; Lane and McDougald, 1982; Pua et al., 1983). For ‘G.30’, the most effective concentration was 2.0 mg·L⁻¹ which increased the number of new shoots and shoot length compared with lower or higher levels. Optimum concentration also depends on the seasonal time when explants are collected with concentrations between 2.0 and 5.0 mg·L⁻¹ being more effective than greater concentrations for ‘Golden Delicious’ explants collected at the start of shoot growth and concentrations below 5.0 mg·L⁻¹ being ineffective for explants collected at the end of the shoot growth phase (Brand, 1993). In our study, 2.0 mg·L⁻¹ BA was consistently the effective for proliferation with explants collected from greenhouse-grown trees in July 2012, and May and June 2013.

The effect of GA₃ on shoot elongation was small compared with other factors such as explant date, chilling, and cytokinin, and was dependent on the concentration of BA. GA₃ increased shoot elongation when combined with the optimum BA concentration of 2.0 mg·L⁻¹. In addition, high GA₃ concentration was detrimental to multiplication indicating that it was supraoptimal at 4.0 mg·L⁻¹. In determining an effective dose of EBR as a media constituent in ‘G.30’, we found both doses 0.1 and 1.0 mg·L⁻¹ to be inhibitory to shoot elongation. In contrast, Engelmann-Sylvestre and Engelmann (2013) found a media concentration of 0.05 mg·L⁻¹ slightly increased shoot elongation in yam. In C. annuum, increase in shoot elongation at this dose is inconsistent, cultivar dependent, and sometimes inhibitory when combined with cytokinin (Franck-Duchenne et al., 1998). Additional work is needed to refine the use of EBR for promoting shoot elongation in apple micropropagation.

Initial shoot multiplication and elongation were highly variable between the two genotypes, experimental treatments, and in vitro conditions. The poor micropropagation efficiency of ‘G.41’ was overcome by an additional month in culture, and was not overcome by a chilling treatment or by increasing GA₃ concentration in the media. ‘G.30’ also demonstrated poor efficiency when explants were collected in summer or fall compared with spring. This was overcome somewhat by chilling explants for 6 weeks. Type of cytokinin and its concentration had a large effect on shoot growth with BA being the most effective type and at a concentration of 2.0 mg·L⁻¹. The plant growth regulator GA₃ had little effect on shoot growth, and EBR was inhibitory at concentrations tested in this study.

**Literature Cited**


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