The Influence of BA and NAA on Adventitious Bud Formation in Leaf Cuttings of Begonia x tuberhybrida

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Keywords: hybrid tuberous begonia, propagation, plant growth regulators, vegetative propagation

Abstract

Begonia x tuberhybrida is propagated mainly by seed, because vegetative propagation by cuttings or tissue culture is difficult. However, seedlings generally exhibit a wide range of variation due to their high level of heterozygosity, and also the pollination period is limited. An adequate technique of vegetative propagation of B. x tuberhybrida if established, would be useful for commercial production. Therefore, we investigated the propagation characteristics of the stem and leaf cuttings, and influence of BA and NAA on adventitious bud formation in leaf cuttings of B. x tuberhybrida.

Stems (2 cm in length) with 5 cm of basal part of leaf blades but with axillary buds removed, and leaf pieces (2.5 x 2.5 cm) cut from young expanded leaves were planted in a mixture of vermiculite and perlite on a bench in a glasshouse controlled at 25/17°C (DT/NT). Both of the stems and the leaf pieces formed only roots or calli, but none of them formed any adventitious buds.

Effects of plant growth regulators on adventitious bud formation were examined by planting leaf pieces (2 x 1.5 cm) on agar medium containing NAA (0-0.5 or 1 ppm) and/or BA (0, 0.5 or 1 ppm) at 23°C. When leaf pieces were planted on agar medium without plant growth regulators, the leaf piece did not form adventitious buds. On the medium with BA, adventitious buds were formed. The leaf pieces planted on the medium with 1 ppm BA formed adventitious buds in 25% and callus in 31% of them. Most leaf pieces in the medium containing NAA died.

BA may be effective for inducing adventitious bud formation in leaf cuttings of B. x tuberhybrida.

INTRODUCTION

Begonia x tuberhybrida belongs to the Begoniaceae family. They have large, flattish perennial tubers depressed at their tops. Their ancestors are seven tuberous species that inhabit the Andes of South America. They have various plant forms, flower sizes and colors, and are one of the most popular pot flower crops.

B. x tuberhybrida plants are propagated mainly by seed, because vegetative propagation with cuttings is difficult. However, seedlings generally exhibit a wide range of variation due to their high level of heterozygosity, and also the pollination period is limited. Although Iida et al. (1986), Peck and Cumming (1984), Viseur and Lievens (1987) and Nakano et al. (1999) developed systems for in vitro propagation of B. x tuberhybrida, the systems are not practical because of the presence of endogenous bacteria in tissue. Most of the research on vegetative propagation of B. x tuberhybrida has been on in vitro propagation. If an adequate technique of vegetative propagation of B. x tuberhybrida in a glasshouse were established, it would be useful for commercial production. In addition, it may allow large-scale propagation from a single plant, and variation due to genetic heterogeneity may be avoided.

Heide (1965) reported that in B. x cheimantha leaf cuttings, cytokinins at a relatively high concentration stimulated bud formation and inhibited root formation. Auxins at the same concentration had the opposite effect. In B. x tuberhybrida also
application of cytokinins to leaf pieces may promote a formation of adventitious buds.

Therefore, we examined the influence of auxin and cytokinin on the formation of adventitious buds on leaf cuttings of *B. x tuberhybrida*.

**MATERIALS AND METHODS**

**Formation of Adventitious Buds on the Stem and Leaf Cuttings from Different Species or Cultivars**

*B. x tuberhybrida* ‘Tenerra Pink’ and ‘Panorama Yellow’ plants grown from seed were used. Stems (nodes, 2 cm long) with the basal 5 cm of the leaf blade but with axillary buds removed (Fig. 1) were used as stem cuttings. Young expanded leaves were also detached from the cuttings. After they were washed with water for a few minutes, leaf pieces (2.5 x 2.5 cm) including a major vein were excised (Fig. 1). The two types of cuttings were planted in cell trays (72 cells) containing perlite and vermiculite on a bench in a glasshouse controlled at 25/17°C (DT/NT) on 31 August, and then they were irrigated with 0.1% thiophanate-methyl solution to prevent rot. To compare its morphogenesis with that of rex begonia, we planted 2.5 x 2.5 cm leaf pieces of *B. rex-cultorum* ‘Princess of Hanover’ by the same method. All cuttings were irrigated to maintain the moisture of the propagation medium. Ten stem cuttings and ten leaf cuttings were used. Eight weeks after planting, numbers of surviving leaf pieces, adventitious buds, roots and calli formed were counted.

**Effects of NAA and BA on the Formation of Adventitious Buds on Leaf Cuttings of *B. x tuberhybrida***

*B. x tuberhybrida* ‘Clips’ plants grown from seed were used. Young expanded leaves were detached and washed with water for an hour. Leaf pieces (2 x 1.5 cm) including thick veins (Fig. 2) were cut and planted on 1% agar medium containing NAA (0, 0.5 or 1 ppm) and BA (0, 0.5 or 1 ppm) in 90 x 20 mm petri dishes and incubated at 23°C (16-hr photoperiods, 115 µmol m−2 sec−1) from 22 October. Thirty-two leaf pieces were used. The leaf pieces nine weeks after planting were examined for numbers of leaf pieces survived, adventitious buds, roots and calli formed at the base of the leaf pieces.

**RESULTS**

**Formation of Adventitious Bud on the Stem and Leaf Cuttings from Different Species or Cultivars**

When node cuttings were planted, the surviving percentage of *B. x tuberhybrida* ‘Tenerra Pink’ and ‘Panorama Yellow’ was 100% but the percentage of adventitious bud formation was 0% (Table 1). Rooting was poorer in *B. x tuberhybrida* ‘Panorama Yellow’ than in *B. x tuberhybrida* ‘Tenerra Pink’.

The surviving percentages of the leaf pieces of *B. x tuberhybrida* ‘Tenerra Pink’, ‘Panorama Yellow’ and *B. rex-cultorum* ‘Princess of Hanover’ were 90%, 60% and 90%, respectively (Table 2). *B. x tuberhybrida* ‘Panorama Yellow’ tended to perish and formed roots in only 40% of the leaf pieces.

Ninety percent of the leaf pieces of *B. rex-cultorum* ‘Princess of Hanover’ formed adventitious buds, but leaf pieces of both cultivars of *B. x tuberhybrida* did not form adventitious buds at all, although some of them formed roots and/or calli.

**Effects of NAA and BA on the Formation of Adventitious Buds on Leaf Cuttings of *B. x tuberhybrida***

Surviving percentage was decreased with increasing concentration of NAA (Table 3). Most leaf pieces perished at the highest NAA concentration (1 ppm). On the other hand, by adding 1 ppm BA, the surviving percentage was increased, up to about 60%, in the absence of NAA.

Leaf pieces planted on the medium without plant growth regulators did not form
adventitious buds. The leaf pieces planted on the medium containing 1 ppm BA formed adventitious buds in 25% and calli in 31% of them.

DISCUSSION

In this study, we investigated the propagation characteristics of stem and leaf cuttings and the effects of plant growth regulators on adventitious bud formation on leaf pieces of *B. x tuberhybrida* in order to establish a method of propagation with leaf cuttings.

The surviving percentage of cuttings was higher with stems than that of leaf cuttings. This may be because leaf pieces were thin and the cut end of the sections were wider, the leaf pieces tended to perish. Most of the whole leaf cuttings survived (data not shown).

In this experiment, all surviving leaf pieces formed adventitious buds in *B. rex-cultorum* ‘Princess of Hanover’. Generally, it is well known that rhizomatous begonia plants including *B. rex-cultorum* easily form adventitious buds and roots on leaf cuttings (Hudson et al., 1997). On the other hand, stem and leaf cuttings of both cultivars of *B. x tuberhybrida* did not form adventitious buds at all, forming roots and/or calli alone.

When leaf pieces were planted on agar medium without any plant growth regulators, none of them formed adventitious buds as on the mixture of perlite and vermiculite.

Peck and Cumming (1984) and Nakano et al. (1999) reported a method for in vitro propagation of *B. x tuberhybrida*. Adventitious buds were formed on leaf pieces (2 x 2 cm) planted on a semisolid MS medium containing 1 ppm NAA and 5 ppm BA (Peck and Cumming, 1984), and on leaf pieces (7 x 7 mm) in a liquid MS medium containing 0.1 ppm NAA and 0.1 ppm BA (Nakano et al., 1999). On the other hand, Heide (1965) immersed the petioles of *B. x cheimantha* in solutions containing plant growth regulators at relatively high concentrations before they planted them in the perlite/peat substrate and succeeded in producing adventitious buds. In our experiment, NAA and BA at 0 to 1 ppm were added to the agar medium, and leaf cuttings were planted on the agar medium. As a result, it became clear that BA induces adventitious bud formation on the leaf cuttings.

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Most leaf pieces died on the agar medium containing NAA. The reason why NAA caused the death of the leaf pieces is unknown, but application of NAA after adventitious bud formation might prevent death and induce rooting. It has also been reported that BA had a protective effect against death. Heide (1965) also found that kinetin had a protective effect against *Botrytis* infection in leaf cuttings of *B. semperflorens-cultorum*.

Thus, BA may be effective for inducing adventitious buds in leaf cutting of *B. x tuberhybrida*.

**Literature Cited**


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### Table 1. Formation of adventitious buds on the stem cutting of *B. x tuberhybrida*.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Surviving percentage</th>
<th>Percentage of organ formation</th>
<th>Adventitious buds</th>
<th>Roots and calli</th>
<th>Only calli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tenerra Pink</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Panorama Yellow</td>
<td>100</td>
<td>0</td>
<td>40</td>
<td>60</td>
<td>0</td>
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</table>

Stems with axillary buds removed and with 5 cm of basal part of leaf blades were planted on a mixture of perlite and vermiculite on a bench in a glasshouse controlled at 25/17°C (DT/NT) on 31 August. After eight weeks organ formation was assessed. n=10 stems.

### Table 2. Formation of adventitious buds on leaf cuttings of *B. x tuberhybrida* and *B. rex-cultorum*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cultivar</th>
<th>Surviving percentage</th>
<th>Percentage of organ formation</th>
<th>Adventitious buds</th>
<th>Roots and calli</th>
<th>Only calli</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. x tuberhybrida</em></td>
<td>Tenerra Pink</td>
<td>90</td>
<td>0</td>
<td>3.1</td>
<td>9.4</td>
<td>18.8</td>
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<tr>
<td></td>
<td>Panorama Yellow</td>
<td>60</td>
<td>0</td>
<td>9.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>B. rex-cultorum</em></td>
<td>Princess of Hanover</td>
<td>90</td>
<td>90</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Leaf pieces (2.5 x 2.5 cm) of *B. x tuberhybrida* and *B. rex-cultorum* were planted in a mixture of perlite and vermiculite in a glasshouse controlled at 25/17°C (DT/NT) on 31 August. After eight weeks organ formation was determined. n=10 leaf pieces.

### Table 3. Effects of NAA and BA on the formation of adventitious buds in leaf cuttings of *B. x tuberhybrida*.

<table>
<thead>
<tr>
<th>Plant growth regulator (ppm)</th>
<th>Surviving percentage</th>
<th>Percentage of organ formation</th>
<th>Adventitious buds</th>
<th>Only roots</th>
<th>Only calli</th>
<th>No change</th>
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<tbody>
<tr>
<td>NAA</td>
<td>BA</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>31.3</td>
<td>0</td>
<td>3.1</td>
<td>9.4</td>
<td>18.8</td>
</tr>
<tr>
<td>0</td>
<td>0.5</td>
<td>25.0</td>
<td>3.1</td>
<td>3.1</td>
<td>18.8</td>
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<tr>
<td>0</td>
<td>1</td>
<td>59.4</td>
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<td>31.2</td>
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<tr>
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<td>0</td>
<td>9.4</td>
<td>0</td>
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<td>9.4</td>
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<td>0.5</td>
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</tr>
<tr>
<td>1</td>
<td>1</td>
<td>3.1</td>
<td>0</td>
<td>3.1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Leaf pieces (2 x 1.5 cm) of *B. x tuberhybrida* were planted on agar medium containing NAA and BA at 23°C on 22 October. After nine weeks organ formation was assessed. n=32 leaf pieces.
Figures

Fig. 1. Stem and leaf cuttings used for the experiments in a glasshouse. Stems (2 cm) with axillary buds removed but with 5 cm of leaf blade (above), and the leaf piece (2.5 x 2.5 cm) cut from young expanded leaves (below).

Fig. 2. Leaf cuttings used for the in vitro experiment. Leaf pieces (2 x 1.5 cm) were cut from young expanded leaves.