Shoot induction and plant regeneration from receptacle tissues of *Lilium longiflorum*

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Abstract

An efficient system has been developed for the in vitro plant regeneration of *Lilium longiflorum* Thunb. by culturing receptacle sections from flower buds. The sections were cultured on one-half MS medium plus 30 g l\(^{-1}\) sucrose, 8 g l\(^{-1}\) agar, 5.4 \(\mu\)M NAA or 4.9 \(\mu\)M IBA plus 2.2 \(\mu\)M BAP. A section size of 3–4 mm was found to be optimal. After 60 days an average of 41 shoots were formed per explant. More vigorous shoots were obtained by subculturing on hormone-free medium with 20 g l\(^{-1}\) sucrose. Rooting occurred on one-half MS medium with 1.1 \(\mu\)M NAA. Rooted plants were hardened-off in a greenhouse for two months, and normal flowering plants were produced. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Flower bud; Section size; Thin cell layer; Vigorous shoot

1. Introduction

*Lilium longiflorum* has been the object of extensive studies for plant regeneration from bulb-scales (Gupta et al., 1978; Nightingale, 1979; Stimart

\begin{center}
\textit{Abbreviations:}  BAP, 6-benzyladenine; IAA, indole-3-acetic acid; IBA, indole butyric acid; MS, Murashige and Skoog; NAA, naphthaleneacetic acid
\end{center}
and Ascher, 1981), leaves (Stenberg et al., 1977), anthers (Qu et al., 1988), stem segments (Nhut, 1998) or embryos (McRae and McRae, 1979). In previous studies on the micropropagation of Lilium species, bulb-scales were shown to be the more favorable explant source for the production and formation of bulblets (Stimart and Ascher, 1978; Takayama and Misawa, 1980, 1983; Niimi, 1986; Gerrits and De Klerk, 1992; Priyadarshi and Sen, 1992; Wickremesinhe et al., 1994; Tanimoto and Matsubara, 1995). Flower stalk fragments of lily (Bigot, 1974) produced adventitious buds and bulblets originating from peripheral tissue.

In the present report we describe factors influencing the regeneration of shoots by using explants from receptacles of L. longiflorum flowers.

2. Materials and methods

2.1. Plant materials

Closed flower buds, 8 cm in length, of L. longiflorum Thunb. ‘nellie white’ were washed thoroughly under running tap water for 30 min, soaked in detergent (Viso, Dong nai, Vietnam) for 5 min, rinsed six times with distilled water, and then with ethanol (70%) for 40 s. After being washed again with distilled water, the closed flower buds were disinfected with a 0.1% aqueous solution of HgCl₂ for 5 min, then rinsed six times in sterile distilled water. The flower stalk, receptacle, folded inner petal, outer petal sheath, ovary, stigma, stamen and anther of closed flower buds were separated out and cut into transversal sections (length is 5 mm).

2.2. Experimental design

2.2.1. Effect of all parts of flower on shoot regeneration

All parts of flower buds (n=40) were cultured on one-half MS medium (Murashige and Shoog, 1962) containing 30 g l⁻¹ sucrose, 8 g l⁻¹ agar (Wako Pure Chemical Industries, Japan) and supplemented with auxin (0 or 5.4 μM NAA, 0 or 4.9 μM IBA, 0 or 5.7 μM IAA) and cytokinin (0, 0.9 or 2.2 μM BAP) for testing the possibility of regeneration of all parts of the flower bud.

2.2.2. Effect of explant thickness on shoot regeneration

Receptacles 1–4, or 5 mm in length were used to test the effect of size on optimal regeneration. These explants were cultured on one-half MS medium containing 30 g l⁻¹ sucrose, 8 g l⁻¹ agar and supplemented with auxin (either 5.4 μM NAA, 4.9 μM IBA, or 5.7 μM IAA) and cytokinin (2.2 μM BAP). Initially, 3-mm long explants (n=40) were used. Explants yielding the highest number of shoots were subsequently used as source of material (shoot clusters) for these experiments.
2.2.3. Effect of sucrose concentration on shoot regeneration

The effect of sugar was examined on the development of vigorous, clonal shoots. Shoot clusters \((n=40)\) were subcultured on one-half MS medium containing different sucrose concentrations \((0, 10, 20 \text{ or } 30 \text{ g l}^{-1})\).

2.3. Culture condition

All multiplication experiments were carried out in borosilicate test tubes \((25 \text{ mm} \times 150 \text{ mm})\), while for rooting, vigorous shoots were cultured in 150-ml flasks on one-half MS medium plus 1.1 \(\mu\)M NAA for 45 days. Each test tube and flask contained 15 or 20 ml, respectively, of medium and was capped with double aluminum foil. Media were adjusted to pH 5.8 before autoclaving at 121°C at 105 KPa for 15 min. All cultures were incubated at 25±1°C with a 12 h photoperiod per day at quantum flux density of 40 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) from fluorescent lamps.

For each treatment, at least 40 explants were used and the experiment repeated three times. Data were recorded at 45, 60 and 90 days after culture. The resulting data from treatments are presented as means with standard errors.

2.4. Transplantation

Fifty shoots were rooted as described previously by culturing on one-half MS medium with 1.1 \(\mu\)M NAA (Nhut, 1998). Plantlets with well-developed roots were removed from the culture vessels and after having their roots washed in running tap water, were transferred to pots containing soil with N/P/K \((10:10:10)\). A plastic cover was inverted over each plantlet to ensure high humidity during the first few days after transfer. Subsequently, the plants were transferred to field conditions.

3. Results

The objective of such a series of experiments is to obtain uniform and vigorous shoots. Since no shoots or roots occurred from explants on media without hormones, and since shooting and rooting occurred together in media containing a single auxin or cytokinin, data for these are not shown since the latter was not desired.

Explants were excised from several parts of \(L. \text{longiflorum}\) flower buds and placed on one-half MS medium supplemented with various combinations of auxins and cytokinin in order to examine which part of the flower bud might form shoots. Of all the explants tested, receptacles had the highest rate of shoot production with 100% survival rate (Table 1). Most explants, except those from...
ovaries, died within 45 days culture (data not shown). Ovary explant tissues enlarged slightly, but did not form shoots on the culture medium (Fig. 1D). In the presence of 4.9 μM IBA or 5.4 μM NAA, the number of shoots per explant was slightly higher than that in media with 5.7 μM IAA and 2.2 μM BAP.

The data on rooting shown in Table 1 indicate that NAA and IBA are equally good in inducing roots while the use of IAA is less efficient. While these experiments provided data on the optimum conditions of media, another set of

<table>
<thead>
<tr>
<th>Growth regulator (μM)</th>
<th>Number of shoots (mean±S.E.)</th>
<th>Number of roots (mean±S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA IBA IAA BAP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.4 0 0 2.2</td>
<td>17.4 ± 3.9</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>5.4 0 0 0.9</td>
<td>14.6 ± 3.3</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td>0 4.9 0 2.2</td>
<td>17.1 ± 3.8</td>
<td>2.5 ± 0.6</td>
</tr>
<tr>
<td>0 4.9 0 0.9</td>
<td>14.4 ± 3.2</td>
<td>4.0 ± 0.9</td>
</tr>
<tr>
<td>0 0 5.7 2.2</td>
<td>16.1 ± 3.6</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>0 0 5.7 0.9</td>
<td>12.9 ± 2.8</td>
<td>1.0 ± 0.6</td>
</tr>
</tbody>
</table>

Table 2
Effects of explant size (n=40) on the regeneration of receptacle sections of L. longiflorum flower buds on three separate media: I, II, III

<table>
<thead>
<tr>
<th>Medium containing 2.2 μM BAP</th>
<th>Size of explants (mm)</th>
<th>Time (days)</th>
<th>Survival rate (%)</th>
<th>Number of shoots per surviving explant (mean±S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+5.4 μM NAA (Medium I)</td>
<td>1</td>
<td>45</td>
<td>15</td>
<td>10.6 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>45</td>
<td>85</td>
<td>30.6 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>60</td>
<td>100</td>
<td>41.4 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>60</td>
<td>100</td>
<td>35.1 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>90</td>
<td>100</td>
<td>18.3 ± 0.9</td>
</tr>
<tr>
<td>+4.9 μM IAA (Medium II)</td>
<td>1</td>
<td>45</td>
<td>15</td>
<td>9.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>45</td>
<td>80</td>
<td>25.0 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>60</td>
<td>100</td>
<td>38.6 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>60</td>
<td>100</td>
<td>33.8 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>90</td>
<td>100</td>
<td>17.6 ± 0.8</td>
</tr>
<tr>
<td>+5.7 μM IAA (Medium III)</td>
<td>1</td>
<td>45</td>
<td>15</td>
<td>7.9 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>45</td>
<td>80</td>
<td>19.9 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>60</td>
<td>100</td>
<td>31.9 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>60</td>
<td>100</td>
<td>25.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>90</td>
<td>100</td>
<td>17.2 ± 0.7</td>
</tr>
</tbody>
</table>
Fig. 1. The growth and development of shoots derived from receptacle section culture: (A) top view of shoots regenerated from receptacle culture (3 mm in length) on medium containing 5.4 μM NAA and 2.2 μM BAP after 60 days culture (bar: 1 mm); (B) side view of shoots regenerated from receptacle culture (3 mm in length) on medium containing 5.4 μM NAA and 2.2 μM BAP after 60 days culture (bar: 1 mm); (C) formation of shoots, aerial roots and calli on 3 mm receptacle sections on various media (bar: 1 mm) (Table 1); (D) ovary section explant showing enlargement on various media (bar: 1 cm) (Table 1); (E) shoots derived from receptacle culture (3 mm in length) induced from medium containing 5.4 μM NAA and 2.2 μM BAP before subdivision (bar: 1 cm) (60 days culture); (F) plantlets formed on rooting medium containing 1.1 μM NAA after 45 days culture (bar: 1 cm).
tests was designed to optimize the size of explants for shoot production. In tests with receptacle explant of varying sizes (1–5 mm), higher number of shoots were obtained in the 3 and 4 mm range (Table 2, Fig. 1A and B). After 60 days in culture the survival rate of the shoots was 100%.

To induce a further development of shoots, shoot clusters were cultured on one-half MS media containing 10, 20 and 30 g l\(^{-1}\) of sucrose, respectively. The media contained no hormones. The culture period was 60 days. Data obtained (Table 3) show that shoots were slightly sturdier when 20 g l\(^{-1}\) was used. When no sugar was used, the shoots became necrotic (100%). Subculturing of the initial shoot clusters on rooting medium enhanced root formation (Fig. 1E as compared to Fig. 1F). In the presence of 30 g l\(^{-1}\) there was slight callusing while at 10 g l\(^{-1}\) the shoots tended to become necrotic, indicating that 20 g l\(^{-1}\) would be the optimum for shoot development.

4. Discussion

Veron et al. (1995) showed that NAA is the most effective auxin for inducing shoot formation in vitro from flower buds and that a cytokinin is essential. From the data presented in this report, it can be observed that the in vitro shoot regeneration of *L. longiflorum* is shown to occur with a high efficiency using receptacle explants on media with NAA or IBA and without passing through a callus phase. The results also suggested that the tissue might have had a relatively high endogenous concentration of cytokinins, since shoot formation occurred in the presence of NAA and IBA and appeared to be independent of the addition of BAP (0 \(\mu\)M, data not shown). High endogenous cytokinin activity has also been shown to be expressed in the induction process of adventitious shoots in transverse thin cell layer explants of *Amaranthus edulis* (Bui et al., 1998).

The stimulating effect of sucrose on shoot formation has also been shown in other lily species (Takayama and Misawa, 1980; Gerrits and De Klerk, 1992; Bonnier and van Tuyl, 1997). However an increase in sucrose concentrations from 6 to 9% reduced shoot growth in *L. longiflorum* (Bonnier and van Tuyl, 1997).
The importance of explant size is in accordance with the results of Silvertand et al. (1992) who showed that 5 mm segments excised from 10 cm long flower stalks of *Allium ampeloprasum* L. formed more than 40 shoots per explant without callus formation. In our studies with *L. longiflorum*, receptacle sections, 3 mm in length, excised from flower buds produced up to 41 shoots per explant, thus indicating that shoot formation does not only depend on hormone type but also on explant size.

In conclusion we have established a method for direct shoot regeneration in *L. longiflorum* Thunb. without an intermediate callus phase using receptacle explants, thus limiting the possibility of obtaining somaclonal variation. This method has already been successfully applied to *L. longiflorum* Thunb. ‘ace’ and *Lilium auratum* Lindl.

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**References**


