Rapid Enlargement of Lily Bulblet by Bioreactor Culture

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Abstract
This study was conducted to investigate the optimal culture condition of lily bulblets in a bioreactor. Asiatic hybrid ‘Mirr’ was used to analyze the effects of growth regulator, sucrose concentration, and charcoal on the endogenous contents of ABA, t-zeatin, starch, and starch accumulation in bulblets. Treatment with growth regulator, BA 0.1 mg L-1 and IAA 0.1 mg L-1, resulted in the highest bulblet proliferation in liquid culture with 5.8 bulblets per scale. Addition of 6% sucrose with 0.1% charcoal to the MS medium showed three times higher bulblet enlargement with 8.4 g per bulblet, compared to 3% sucrose alone at 2.7 g/bulblet. Starch content was higher with 9% sucrose with 0.1% charcoal, and accumulation of starch in the cell showed subsequent increases with sucrose concentration in the medium. The starch accumulation was higher in solid agar medium than in the liquid medium. ABA content was lower at 3% sucrose, than other concentrations. This was probably due to a relationship between the bulblet enlargement and endogenous ABA content. The t-zeatin content was highest in the 6% sucrose and 0.1% charcoal treatment. A liquid culture method using an Ebb & flow system resulted in better bulblet enlargement, especially in 6% sucrose treatments, than the ordinary liquid culture method.

INTRODUCTION
Lily is cultivated over the world as a high value cut flower ornamental crop. It is propagated by scaling in the field and by mass and micro propagated tissue culture in vitro (Kim et al., 1996, 2002). A variety of in vitro methods have been used to culture lily of in vitro associated with the production of virus free plants (Niimi et al., 1999, 2001, 2002; Xu et al., 2000). In vitro culture of lily has been conducted on solid culture with MS medium, but recently a number of crops have been cultured on liquid medium with MS basal medium (Hahn et al., 2003). Although changes in several endogenous materials have been studied during the in vitro culture of lily (Shin et al., 2002), the production of lily bulblets via bioreactor is not well established.

This study was conducted to investigate the optimal culture conditions for lily bulblets enlargement and to analyze changes in endogenous materials of bulblets in liquid culture using a bioreactor.

MATERIALS AND METHODS
Asiatic hybrid lily ‘Mirr’ was used as plant material and scales were cultured in solid or liquid MS basal medium in vitro. Culture conditions of the tissue culture room were 25°C with 16 hours day length. For growth and bulblet formation, 0 to 0.5 mg L-1 of BA and 0.1 to 1.0 mg L-1 of IAA were added to the MS media. Active charcoal (0.1%) and 3% to 9% sucrose were added for bulblet enlargement. An Ebb & flow system was used with the bioreactor. The bioreactor volume was 5 L. Endogenous t-zeatin and ABA analysis was conducted as described by Sanyal and Bangerth (1998) with competitive ELISA analysis. Sucrose and starch contents were analyzed by HPLC as described by Ding et al. (1998) and Chaplin (1986) and starch accumulation in a cell was monitored for each of the sucrose concentration and active charcoal treatments. Samples were fixed in FAA and dehydrated in an ethanol series and embedded in paraffin. Sections were cut using a rotary microtome and double-stained with PAS and observed under a light microscope.
RESULTS AND DISCUSSIONS

The treatment with growth regulator, BA 0.1 mg L\(^{-1}\) and IAA 0.1 mg L\(^{-1}\), resulted in the highest bulblet proliferation in liquid culture with 5.8 bulblets per scale. For bulblet weight, BA 0 mg L\(^{-1}\) and IAA 0.5 mg L\(^{-1}\) treatment was more effective than other rates (Table 1).

In the bulblet growth treatments involving different sucrose concentrations and active charcoal, the treatment with 6% sucrose with 0.1% charcoal resulted in three times higher bulblet enlargement (8.4 g per bulblet) compared to 3% sucrose (2.7 g per bulblet). Sharma and Kanwar (2003) found that formation of daffodil bulblets was greater at high sucrose concentration. The addition of active charcoal increased enlargement of bulblet (Table 2).

Starch content was higher in 9% sucrose with 0.1% charcoal (320 mg/g F.W.), and the total amount of starch was more than sucrose alone. The addition of active charcoal also increased starch levels (Fig. 1). Total sucrose of bulblets increased during long term storage with low temperature and starch decrease (Shin et al., 2002). In our histological study, accumulation of starch in the cell increased with increased sucrose concentration in the medium. The starch accumulation was higher in solid agar medium than in the liquid medium. Active charcoal didn’t affect the accumulation of starch (Fig. 2).

ABA content was lower at 3% sucrose than at other sucrose concentrations. This was probably due to a relationship between the bulblet enlargement and endogenous ABA content. The higher amount of endogenous ABA, the more weight of bulblet. There was a much higher ABA content at higher concentrations of sucrose. The amount of ABA was 1.8 mg/g F.W. at 3% sucrose and about 7 mg/g F.W. at 6% (Fig. 3).

In the analysis of endogenous cytokinin, t-zeatin content was highest in the 6% sucrose and 0.1% charcoal treatment. This was considered to be an endogenous cell division substance (Fig. 4). The liquid culture method using the Ebb & flow system resulted in better bulblet enlargement, especially at 6% sucrose, than the ordinary liquid culture method. This was probably the result of better aeration than during liquid culture. Fresh weight of bulblets using the Ebb & flow system was two times higher than liquid culture (Fig. 5).

Literature Cited


Scientia Hort. 97:57-63.

**Table 1. Growth and bulblet formation of lily ‘Mirr’ at different BA and IAA concentrations (mg L⁻¹).**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bulblet weight (mg)</th>
<th>No. of bulblets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (MS+3% sucrose)</td>
<td>110.5c⁰</td>
<td>3.0b</td>
</tr>
<tr>
<td>BA 0  IAA 0.1</td>
<td>197.2b</td>
<td>3.8b</td>
</tr>
<tr>
<td>BA 0.5</td>
<td>282.7a</td>
<td>3.8b</td>
</tr>
<tr>
<td>BA 1.0</td>
<td>209.6b</td>
<td>3.2b</td>
</tr>
<tr>
<td>BA 0.1  IAA 0.0</td>
<td>84.6c</td>
<td>2.6b</td>
</tr>
<tr>
<td>BA 0.5  IAA 0.0</td>
<td>246.8ab</td>
<td>5.8a</td>
</tr>
<tr>
<td>BA 1.0  IAA 0.1</td>
<td>192.7b</td>
<td>3.7b</td>
</tr>
<tr>
<td>BA 1.0  IAA 0.5</td>
<td>230.0ab</td>
<td>4.5ab</td>
</tr>
</tbody>
</table>

⁰Mean separation within columns by Duncan’s multiple range test at P=0.05.

**Table 2. Fresh weight and number of bulblets of lily ‘Mirr’ cultured at different liquid medium for 5 months.**

<table>
<thead>
<tr>
<th>Medium (liquid)</th>
<th>Fresh wt. of bulblet (g)</th>
<th>No. of bulblets</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS+3% sucrose</td>
<td>2.7b⁰</td>
<td>2.2b</td>
</tr>
<tr>
<td>MS+6% sucrose</td>
<td>6.2ab</td>
<td>1.6ab</td>
</tr>
<tr>
<td>MS+9% sucrose</td>
<td>3.1b</td>
<td>0.4c</td>
</tr>
<tr>
<td>MS+6% sucrose+AC⁰</td>
<td>8.4a</td>
<td>0.8b</td>
</tr>
<tr>
<td>MS+9% sucrose+AC²</td>
<td>4.9b</td>
<td>0.2c</td>
</tr>
</tbody>
</table>

⁰Mean separation within columns by Duncan’s multiple range test at P=0.05.

⁲active charcoal
Figures

Fig. 1. Endogenous content of sucrose and starch in lily ‘Mirr’ bulblet cultured by bioreactor and solid medium for five months.

Fig. 2. Accumulation of starch in a lily ‘Mirr’ bulblet cell at different sucrose concentrations and the addition of active charcoal (AC).
Fig. 3. Endogenous content of ABA in lily ‘Mirr’ bulblet cultured by bioreactor and solid medium.

Fig. 4. Endogenous content of t-zeatin in lily ‘Mirr’ bulblet cultured by bioreactor and solid medium.

Fig. 5. Comparison of fresh weight between Ebb & flow system and liquid culture. Modified MS basal medium was used in each treatment.