Tissue Culture Propagation of Liriope muscari and Ophiopogon jaburan

John J. Frett and Michael A. Dirr
Department of Horticulture, University of Georgia, Athens, GA 30602

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Abstract. Inflorescence explants of Liriope muscari Bailey 'Variegata' and Ophiopogon jaburan Lodd. 'Variegatus' produced multiple shoots in vitro on a modified Murashige and Skoog medium via callus. In subsequent studies with L. muscari 'Variegata', there was no difference in floret and scape explant growth, while rhizome and modified root explants were either contaminated or failed to grow. Distal floret explants produced more shoots than proximal explants but there were no significant positional differences in scape explants. In liquid culture 2,4-dichlorophenoxyacetic acid (2,4-D) at 0.5 mg/liter promoted the greatest fresh weight and shoot and root length. Benzylaminopurine (BA) at 0.1 mg/liter increased the number of shoots while decreasing the number of roots. Agitation of liquid cultures increased callus fresh weight.

Many liliaceous plants have been produced successfully through tissue culture. Liriope species have been cultured in vitro utilizing leaf and bulb scale explants grown on modified Murashige and Skoog (MS) media supplemented with various vitamins and growth regulators (4, 9, 11, 13). Generally, the cytokinin BA (2, 4, 9, 14) is used in combination with naphthaleneacetic acid (NAA) (9, 11, 14). Hemerocallis is produced commercially through tissue culture (5). Shoot tips (12), immature inflorescences (1, 6), and sepal and petal (3) explants can be used. Again, MS media supplemented with various organic additives and either kinetin (1, 3, 6) or isopentenyladenine (2iP) (12) with indoleacetic acid (IAA) (1, 3, 12) have been effective with Hemerocallis. Hosta can be propagated rapidly through tissue culture (7, 10) by using immature inflorescences and modified MS media.

The purpose of these experiments was to determine the feasibility of propagating 3 cultivars of Liriope muscari and 2 taxa of Ophiopogon jaburan through tissue culture.

Species and cultivar effects. L. muscari 'Variegata', 'Christmas Tree', 'Monroe White', and O. jaburan and 'Variegatus' inflorescences were harvested when the individual florets were still in tight bud. The smallest floret from each cluster of florets in the inflorescence and 5-mm segments of scape were cut into explants following the sterilization procedure. The 4 explants and 6 positions were replicated 6 times in a completely randomized design. The 4 explants and 6 positions were replicated 6 times in a completely randomized design and incubated as above for 8 weeks.

All of the 36 rhizome and 25 of the modified root explant cultures of L. muscari 'Variegata' were contaminated; modified root cultures that were not contaminated did not grow. Of the floret tissue explants, 45% grew; 20% produced shoots and 10% produced roots. Scape tissue explants survived 39% of the time; 8% of the cultures produced shoots and 5% produced roots. There was no positional effect of 2,4-D and BA concentrations and culture agitation on fresh weight, shoots, and roots of Liriope muscari 'Variegata' callus grown in liquid culture for 8 weeks.

Table 1. Survival of inflorescence explants of Liriope and Ophiopogon after 8 weeks in culture on a modified MS medium with 2 mg IAA and 2 mg kinetin.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>No. surviving cultures</th>
<th>No. cultures producing callus</th>
<th>No. cultures producing shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liriope muscari 'Variegata'</td>
<td>23</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>L. muscari 'Christmas Tree'</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L. muscari 'Monroe White'</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ophiopogon jaburan</td>
<td>24</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>O. jaburan 'Variegatus'</td>
<td>27</td>
<td>16</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 2. Effect of 2,4-D and BA concentrations and culture agitation on fresh weight, shoots, and roots of Liriope muscari 'Variegata' callus grown in liquid culture for 8 weeks.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Callus fresh wt (g)</th>
<th>No. shoots</th>
<th>Shoot length (mm)</th>
<th>No. roots</th>
<th>Root length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.1 a</td>
<td>7.0 a</td>
<td>5.0 a</td>
<td>0 a</td>
<td>0 a</td>
</tr>
<tr>
<td>0.5</td>
<td>3.8 b</td>
<td>3.5 a</td>
<td>23.4 b</td>
<td>5.3 b</td>
<td>8.8 b</td>
</tr>
<tr>
<td>1.0</td>
<td>2.2 ab</td>
<td>1.0 a</td>
<td>3.8 a</td>
<td>3.0 ab</td>
<td>6.2 ab</td>
</tr>
<tr>
<td>BA (mg/liter)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>2.0 a</td>
<td>0.1 a</td>
<td>1.6 a</td>
<td>17.6 b</td>
<td>15.7 a</td>
</tr>
<tr>
<td>0.2</td>
<td>2.8 a</td>
<td>4.0 b</td>
<td>18.1 a</td>
<td>3.8 a</td>
<td>12.4 a</td>
</tr>
</tbody>
</table>

Cultures state

Stable: 1.4 a

Agitated: 3.3 b

Mean separation in columns within treatments by Duncan’s multiple range test, 5% level.

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1Graduate Student.
2Assistant Professor.
In Vitro Propagation of Syringa vulgaris ‘Vesper’

Virginia Hildebrandt1 and Patricia M. Harney2
Department of Horticultural Science, University of Guelph, Guelph, Ont., Canada N1G 2W1

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Abstract. Explants of actively growing shoot tips from greenhouse-grown plants of ‘Vesper’ lilac (Syringa vulgaris L.) initiated new shoots in 2–4 weeks on a modified Murashige and Skoog (MS) revised medium plus 0.1 mg/liter 6-benzylaminopurine (BA) and either 0.125, 0.25, or 0.5 mg/liter indoleacetic acid (IAA). These shoots were transferred for multiplication to the same medium but with 7.5 mg/liter BA and 0.1 mg/liter β-naphthalenacetic acid (NAA). In 5–6 weeks about 6 shoots, 12–15 mm in length, had been produced per explant. There was no increase in the number of shoots by placing them in either a horizontal or inverted position compared to upright. Although excised shoots would root in vitro, rooting was more successful in vermiculite in a plastic-covered flat.

Although the lilac may be propagated by softwood cuttings taken 10–14 days after flowering (13), only 35–60% of some lilac cultivars root successfully. As a result, many cultivars are propagated commercially by grafting onto seedling lilac or privet rootstock (5). The recent success with in vitro techniques in the propagation of a number of woody species (4, 7) suggested the possibility of using such techniques with lilac.

Cultures of ‘Vesper’ lilac were initiated from actively growing shoot tips collected from 1½ to 2-year-old greenhouse-grown plants. Explants, 5- to 10-mm-long, were prepared from these shoot tips by removing the leaves and soaking them for 5–10 minutes successively in 0.5% sodium hypochlorite (10% commercial bleach) with one drop of Tween 20 per 50 ml and 2 times in sterile distilled water. The explants were cultured on solid medium containing MS salts (12) and per liter: 100 mg myo-inositol, 1 mg nicotinic acid, 1 mg pyridoxine HCI, 1 mg thiamine HCl, 30 g sucrose, and 7 g agar at either pH 4.5 or 5.6. Three different concentrations of IAA (0.125, 0.25 and 0.5 mg/liter) and 0.1 mg/liter BA were added to this basic medium. The medium was dispensed in 10-ml aliquots to 25 × 150-mm test tubes and in 15-ml aliquots to 35-mm, square sampling bottles, autoclaved at 1.4 kg/cm2 and 121°C for 15 min, and then cooled at room temperature for 24 hr before use. Shoot tip cultures were incubated at 27 to 28°C with a light intensity of 41 μE s–1 m–2 for 18 hr/day.

After 2–4 weeks in initiation medium, explants produced elongated shoots either from the apex or from lateral buds. The percentage of explants initiating shoots showed little variation, with the least response at 0.5 mg/liter, pH 5.6 (Fig. 1).

Preliminary experiments showed that low BA favored the production of callus, and, as noted by Uhrig (16) and Minocha (11), this callus did not differentiate. About 90% of the samples in a shoot medium with 7.5 mg/liter BA and 0.1 mg/liter NAA had multiple shoots. The mean number of shoots increased with each subculture up to the 4th. There was considerable variation from one subculture to another and among replicates of a subculture. By the 4th subculture, production ranged from 1 to 9 per explant, with shoots smaller than 2 mm in length unable to survive transfer.

Upright or horizontal position of the explants were not important. When placed in a horizontal position, about 60% of shoots remained attached to the shoot tips and were eventually rooted. The rooting rate of this work was compared to rooting rates from softwood cuttings from actively growing shoots collected in the greenhouse, and the results were not significantly different (13).

The adventitious roots were 2–4 mm long, with an average of 2 per shoot. About 80% of the shoots produced roots. Shoots, 12–15 cm long, were taken from cultures that had developed more than 6 shoots and were removed from the subculturing medium to a rooting medium (12). The rooting medium contained Murashige and Skoog basal salts plus 1 mg/liter each of indole-3-acetic acid and IAA, 3% sucrose and 7 g agar (Table 1). The shoot tips were placed in a horizontal position in 125-ml Erlenmeyer flasks containing 20 ml of media. Each treatment was replicated 4 times.

Callus growth, shoot length, and root number and length were highest with 2,4-D at 0.5 mg/liter (Table 2). There was no significant difference in shoot number in response to 2,4-D due to high variability, but the trend suggested that increasing 2,4-D concentration decreased shoot number.

Another experiment was designed to determine the effect of BA at 0 or 0.1 mg/liter and agitation on L. muscari ‘Variegata’ callus growth. The effect of agitation was determined by comparing growth of cultures incubated in a stationary water bath to cultures shaken at 60 opm. 2,4-D was included in the control to 4 and decreased root number from 17.6 to 3.8 (Table 2). BA did not significantly affect callus growth, shoot length, or root length. Agitation of cultures significantly increased the fresh weight from an average of 1.4 to 3.3 g but had no effect on organogenesis compared to those grown in a stationary water bath.


Literature Cited