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Tissue Culture Propagation of *Liriope muscari* and *Ophiopogon jaburan*

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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. Benzylamino purine (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. *Lilium* 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 naphthalenacetic 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 tissue immediately below the lowest cluster of florets were used for explants. Explants were surface-sterilized in 0.5% sodium hypochlorite for 20 min, rinsed 3 times in sterile water, and placed on a modified MS medium (8) containing (per liter): 80 mg adenine sulfate, 100 mg inositol, 170 mg KH_2PO_4, 0.4 mg thiamine hydrochloride, 30 g sucrose (referred to as basal MS medium), 2 mg IAA, and 2 mg kinetin. Thirty-two explants of each taxon were cultured and maintained in incubators at 22°C with a 16-hr photoperiod with 20 $\mu E\ s^{-1}\ m^{-2}$ of fluorescent light and evaluated after 8 weeks.

L. muscari 'Variegata' displayed the greatest propensity for growth in culture compared to the other *Liriope* cultivars (Table 1). Some organogenesis occurred after 8 weeks in culture with 'Variegata' but none with 'Christmas Tree' or 'Monroe White'. Variable organogenesis occurred following subculture of 'Variegata' with 1 to 10 shoots forming per culture. *O. jaburan* produced callus in the primary culture, but no organogenesis occurred even after subculture. *O. jaburan* 'Variegatus' grew vigorously and produced callus and a few shoots. Subculture of the callus yielded multiple shoots, up to 10 shoots per tube. Shoots of *L. muscari* 'Variegata' and *O. jaburan* 'Variegatus' underwent chimeral segregation with production of green, albino, and variegated shoots. Only 10% of the shoots rooted when transferred to a mist propagation bench, but shoots rooted readily in the multiplication medium. Shoots which rooted—as well as those which formed roots *in vitro*—have been established and grown under greenhouse conditions.

Explant effects. In a second experiment, 4 explant sources (rhizome, nodular modified root, floret, and scape) of *L. muscari* 'Variegata' were compared. Inflorescences were divided equally into 6 pieces, and one 5-mm explant was taken from each piece and the positional integrity maintained (position 1, distal, through 6). Similarly, six 5-mm scape explants were taken from 30 mm of scape tissue immediately below the inflorescence. Pieces of rhizome and nodular modified root were washed in soapy water and cut into 6 cross-sectional pieces, 5 mm thick for explants. All explants were surface-sterilized intact and cut into explants following the sterilization procedure. 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

Table 1. Survival and growth 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.

Taxa	No. surviving cultures	No. cultures producing callus	No. cultures producing shoots
Liriope muscari 'Variegata'	23	3	1
L. muscari 'Christmas Tree'	2	0	0
L. muscari 'Monroe White'	1	0	0
Ophiopogon jaburan	24	1	0
O. jaburan 'Variegatus'	27	16	4

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.

Treatment	Callus fresh wt (g)	No. shoots	Shoot length (mm)	No. roots	Root length (mm)
2,4-D (mg/liter)					
0	1.1 a[z]	7.0 a	5.0 a	0 a	0 a
0.5	3.8 b	3.5 a	23.4 b	5.3 b	8.8 b
1.0	2.2 ab	1.0 a	3.8 a	3.0 ab	6.2 ab
BA (mg/liter)					
0	2.0 a	0.1 a	1.6 a	17.6 b	15.7 a
0.1	2.9 a	4.0 b	18.1 a	3.8 a	12.4 a
Culture state					
Stable	1.4 a	0.5 a	10.1 a	11.3 a	13.4 a
Agitated	3.3 b	3.0 a	6.8 a	12.3 a	15.3 a

[z]Mean separation in columns within treatments by Duncan's multiple range test, 5% level.

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effect of the scape explants; however, the distal-most floret explants produced significantly more shoots.

Media effects. L. muscari 'Variegata' callus, obtained from primary cultures, was evaluated in liquid culture using the same basal medium. Cultures were incubated in a shaking water bath at 60 oscillations per min (opm) at 25°C with 5 $\mu E\ s^{-1}\ m^{-2}$ of continuous fluorescent light for 8 weeks with three 2,4-D concentrations (0, 0.5, and 1 mg/liter). About 1 cm^3 of callus was placed in a 125-ml Erlenmeyer flask 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 basal medium at 0.5 mg/liter and the growth environment was the same as described for the 2,4-D experiment.

BA increased the number of shoots from < 1 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.

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In Vitro Propagation of *Syringa vulgaris* 'Vesper'

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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-benzylamino purine (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-naphthaleneacetic 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 HCl, 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/cm^2 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 $\mu E\ s^{-1}\ m^{-2}$ 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 Uhring (16) and Minocha (11), this callus did not differentiate. About 90% of the samples in a shooting 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 ex-

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