at least doubled when the concentration of ammonium nitrate was decreased to 6% or 25% under otherwise standard culture conditions (Table 2). Highest survival for 'Delaware' was obtained at 6% and 25% of full-strength ammonium nitrate and that of 'V. thunbergii' at 6% of full strength. 'Rizamat' survived about equally, regardless of ammonium nitrate concentration. These results suggest that identical storage conditions could be applied to the three grape species using reduced ammonium nitrate concentrations in MS medium.

Almost all of the nodal cultures developed shoots and roots during storage. More shoots proliferated under low concentration of ammonium nitrate (Table 2) than under low temperature (Table 1). All surviving shoots had a healthy green appearance and normal morphogenesis. After transfer to fresh medium, the growth of these shoots was not impaired by previous storage. Barlass and Skene (1) demonstrated that the proliferation of grape cultures was dependent on nutrition; i.e., full-strength MS was better than half-strength. In addition, half-strength MS prevented grape cultures from blasting, compared with full-strength (9). These data suggested that reduced medium was suitable for the enhancement of survival as well as for the depression of growth in grape cultures. Therefore, it seemed that low concentration of ammonium nitrate could reduce growth of cultures with high survival rate.

A method of medium-term storage of grape nodal culture is proposed consisting of standard tissue culture conditions in addition to a low concentration of ammonium nitrate in the medium without subjecting the cultures to low temperature.

### Literature Cited


### Table 2. Percent survival and number of shoots per grape nodal culture after storage in MS medium containing 0%, 6%, 25%, and 100% of full-strength ammonium nitrate. *

<table>
<thead>
<tr>
<th>Species and cultivar</th>
<th>Storage duration (days)</th>
<th>Survival (%) at ammonium nitrate concentration (%) given**</th>
<th>No. shoots at ammonium concentrations (%) given***</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. labrusca</td>
<td>290</td>
<td>60b 70b 70b 30a</td>
<td>1.3 ± 0.6 1.3 ± 0.5 1.2 ± 0.4 1.2 ± 0.4</td>
</tr>
<tr>
<td>Delaware</td>
<td>283</td>
<td>60ab 80b 70ab 35a</td>
<td>1.2 ± 0.4 1.5 ± 0.5 1.5 ± 0.5 1.4 ± 0.5</td>
</tr>
<tr>
<td>V. thunbergii</td>
<td>262</td>
<td>55a 80a 80a 80a</td>
<td>2.2 ± 0.6 2.1 ± 1.0 3.0 ± 1.1 3.1 ± 1.1</td>
</tr>
<tr>
<td>V. vinifera</td>
<td>283</td>
<td>60ab 80b 70ab 35a</td>
<td>1.2 ± 0.4 1.5 ± 0.5 1.5 ± 0.5 1.4 ± 0.5</td>
</tr>
</tbody>
</table>

*Stored under 16-hr photoperiod at 28°C.

**Percentage relative to full-strength Murashige and Skoog medium.

***Contingency X² P = 5%.

****Mean ± sd.

---


In Vitro Shoot Regeneration on Leaf Tissue from Micropropagated Highbush Blueberry

P. Callow, K. Haghhighi, M. Giroux, and J. Hancock
Department of Horticulture, Michigan State University, East Lansing, MI 48824-1325

**Additional index words.** Vaccinium corymbosum, growth regulator, wounding

**Abstract.** Leaf explants from shoot-proliferating cultures of highbush blueberry (Vaccinium corymbosum L.) produced shoots on one-half-strength Murashige and Skoog medium (MS) supplemented with 5, 25, 50, or 100 µmol of 2iP. The highest number of meristematic nodules and buds per leaf were induced at 25 µmol 2iP and the least at 5 or 100 µmol. Nodule, bud, and shoot regeneration was significantly increased by wounding the leaf explants. Chemical name used: 6-(γ-dimethylallylamino)purine (2iP).

It is now possible to transform many higher plants using genetically altered Agrobacterium (Klee et al., 1987). This technology offers exciting possibilities for single gene transfer. A prerequisite to Agrobacterium-mediated transformation is the ability to regenerate whole plants from somatic tissues.

---

**Table 1.** Percentage of meristematic nodules, adventitious buds, and shoots found at different leaf surface locations after 5 weeks.

<table>
<thead>
<tr>
<th>Location</th>
<th>Meristematic nodules (%)</th>
<th>Bud (%)</th>
<th>Shoot (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf margin</td>
<td>5.7</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Midrib</td>
<td>12.0</td>
<td>11.0</td>
<td>28.6</td>
</tr>
<tr>
<td>Wounded area</td>
<td>50.8</td>
<td>83.0</td>
<td>73.0</td>
</tr>
<tr>
<td>Intervenial</td>
<td>1.2</td>
<td>5.7</td>
<td>0.0</td>
</tr>
</tbody>
</table>
such as leaf disks, hypocotyls, or cotyledons. Diverse woody plants have proven amenable to such techniques, including: aspen (Noh and Minocha, 1986), azalea (Fordham et al., 1982), fig (Jonas and Grishuolo, 1987), orange (Kobayashi et al., 1985), mountain laurel (Lloyd and McCown, 1980), pomegranate (Omura et al., 1987), and low-bush blueberry (Nickerson, 1978). However, most reports of tissue culture with the cultivated highbush blueberry have been limited to shoot tip proliferation (Grout and Read, 1986; Lyrene, 1980; Frett and Smagula, 1982) or adventitious buds forming on attached leaves of shoot tip cultures (Zimmerman and Broome, 1980). Only recently, while this manuscript was in review, have reports of successful highbush blueberry regeneration been made (Dweikat and Lyrene, 1988; Billings et al., 1988). In this study, we describe the upper threshold of 2iP, which stimulates shoot formation, and document the value of leaf scoring before placing the explants on medium.

Softwood cuttings were taken from greenhouse-grown plants of 'Bluecrop' highbush blueberry in Oct. 1987. The plant material was dipped in 95% ethanol for 30 sec, followed by a 30-min soak in a 0.6% solution of commercial sodium hypochlorite containing 0.02% Tween 20. This solution was periodically agitated. The tissue was then rinsed three times with sterile deionized water. Single-node stem explants were cut to a length of 1.5 cm and placed on a medium containing one-half-strength Murashige and Skoog (1962) inorganic salts with 0.4 mg thiamine/HCl per liter, 100 mg myo-inositol/liter, 2% sucrose, 0.4% Difco Bacto-agar, 25 μmol 2iP and the pH adjusted to 5.0. Single-node explants were initially placed in culture tubes (150 × 25 mm) under a 16-hr photoperiod at 25 to 40 μmol·s⁻¹·m⁻² (cool-white, fluorescent) and 23 ± 2°C. After 4 weeks, buds developed into shoots that had elongated to ~4 cm. They were then placed horizontally on medium in Magenta GA-7 containers for further shoot proliferation. Proliferating shoots were subcultured every 5 weeks.

In vitro-grown leaves (~5 to 10 mm long) were removed from the proliferating shoots 3 to 5 weeks after subculture and placed in petri dishes (100 × 15 mm) on the same basal Murashige and Skoog medium containing 5, 25, 50, or 100 μmol 2iP. The leaves were lightly scored with a scalpel on the abaxial side and were placed abaxial side down on the medium. The cultures were maintained under the previously described environmental conditions and, after 3 to 4 weeks, the number of meristematic nodules (first stage of bud initiation), adventitious buds, and shoots on each leaf were counted.

Shoots formed without callus formation on the margin, midrib of the lamina, and wounded and interveinal regions of the leaves (Table 1). A gradient in shoot formation was evident on the leaf with the area nearest the wound having the highest concentration of meristematic nodules that gave rise to buds and shoots (Fig. 1A). After 5 weeks, small shoots, ~0.5 cm in length, were visible and could be transferred to proliferation media (Fig. 1B). The shoots proliferated (Fig. 1C) and were easily rooted in soil containing 1 part : 1 perlite : 1 vermiculite (by volume).

There was a significant, negative correlation (r = -0.87) between the number of meristematic nodules per leaf and 2iP concentration, with 25 μmol showing the highest numbers (Table 2). A significant correlation was not observed between 2iP concentration and leaves with shoots or buds per leaf, but, in both cases, the 25-μmol treatment had the highest value. The number of shoots per leaf was significantly correlated (r = 0.92) with 2iP concentration.

Our results demonstrate that high numbers of shoots can be produced on leaf explants maintained on half-strength MS medium supplemented with 5 to 25 μmol 2iP. This system has now been successfully applied in our laboratory to the blueberry cultivars Bluecrop, Bluejay, and Jersey. The regeneration of highbush blueberry from leaf pieces removes the first block for Agrobacterium-mediated transformation of this species.

**Literature Cited**


---

**Table 2. Effect of 2iP concentration on the formation of shoot, bud, and meristematic nodules of in vitro-grown leaves of blueberry after 5 weeks.**

<table>
<thead>
<tr>
<th>2iP concn (μmol)</th>
<th>Total leaves (no.)</th>
<th>Leaves with shoots (no.)</th>
<th>Meristematic nodules per leaf (no.)</th>
<th>Buds per leaf (no.)</th>
<th>Shoots per leaf (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>34</td>
<td>7</td>
<td>4.9 ± a</td>
<td>0.28 ± a</td>
<td>0.57 ± a</td>
</tr>
<tr>
<td>25</td>
<td>34</td>
<td>13</td>
<td>6.7 ± c</td>
<td>4.5 ± c</td>
<td>0.54 ± a</td>
</tr>
<tr>
<td>50</td>
<td>38</td>
<td>7</td>
<td>4.0 ± a</td>
<td>4.2 ± c</td>
<td>0.87 ± b</td>
</tr>
<tr>
<td>100</td>
<td>34</td>
<td>-0.32</td>
<td>-0.87*</td>
<td>-0.46 ± 0.76</td>
<td>0.92*</td>
</tr>
</tbody>
</table>

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

r = Correlation between 2iP concentration and the various traits.

*Significant at the 5% level.
In Vitro Propagation of Lavender

Maria Carmen Calvo and Juan Segura
Departamento de Fisiologia Vegetal, Facultad de Farmacia, Universidad de Valencia, Avda, Blasco Ibañez 13, 46010-Valencia, Spain

Abstract. Cultures were established for micropropagation of Lavandula latifolia Medicus from hypocotyl explants. Bud induction was achieved by placing the explants on Murashige and Skoog (MS) medium supplemented with 0.60 μM IAA and 8.9 μM BA. The buds developed into numerous shoots on transfer to several elongation media. Shoots were easily rooted (95%) on MS hormone-free medium with macronutrients at half-strength. Plantlets were successfully transplanted into soil. Chemical names used: N-(phenylmethyl)-1H-purin-6-amine (BA); 1H-indole-3-acetic acid (IAA).

Lavender [Lavandula latifolia Medicus (Labiatae)] is one of the most important aromatic crops in Mediterranean areas. Although this species can be vegetatively propagated, the poor rooting ability of the stem cutting, as well as the lack of selected clones, restrains its economical exploitation. Thus, tissue culture technology may overcome these limitations by providing methods for rapid multiplication and improvement of this species. Except for a preliminary report on the in vitro multiplication of this species from axillary buds (6), the successful micropropagation of lavender has not been reported. In a previous investigat (3), hypocotyl explants from lavender were found to be highly caulogenic, with the potential for mass propagation. However, most of the induced buds failed to produce well-developed shoots. Efficient micropropagation procedures require not only the production of a large number of shoots, but also continued development until they can be manipulated for rooting and acclimatization. This study was to establish a micropropagation protocol for lavender through adventitious bud formation from hypocotyl explants.

Plant Cell Tissue Organ Cult. 4:249-259.

Received for publication 1 Feb. 1988. This research was supported by The Dirección General de Investigación Cientifica y Técnica, Madrid (Spain), project no. PB86-0066. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.