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# Rapid Multiplication of Veronica 'Red Fox' Propagated in Vitro

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***Abstract.*** **Shoot tips from in vitro cultures of *Veronica spicata* L. 'Red Fox' were grown on modified Woody Plant Medium containing one of 3 cytokinins or cytokinin plus NAA. Of the 3 cytokinins tested (BA, kinetin, and 2iP), BA was the most effective stimulator of axillary shoot growth. The greatest number of shoots greater than or equal to 5 mm in length was produced on medium containing 8 μM BA plus 0.01 μM NAA, whereas medium containing 2 μM BA plus 0.01 μM NAA produced the greatest number of shoots greater than or equal to 10 mm. Shoots were rooted and established at an 80% rate in a soilless medium under high humidity. Chemical names used: *N*-(phenylmethyl)-1*H*-purin-6-amine (BA); *N*-(3-methyl-2-butenyl)-1H-purin-6-amine (2iP); *N*-(2-furanylmethyl)-1*H*-purin-6-amine (kinetin); 1-naphthaleneacetic acid (NAA).**

*Veronica spicata* (spike speedwell) is an ornamental herbaceous perennial grown for its lance shaped leaves and terminal flowers (7). The cultivar 'Red Fox' has clear reddish-pink flowers which are produced freely all season (9). Propagation can be accomplished by dividing clumps in the spring or fall, and softwood cuttings can be rooted during the summer (7). Micropropagation could be used to reduce stock plant maintenance, avoid seasonal propagation restrictions, and increase the multiplication rate. In vitro propagation of some members of the Scrophulariaceae (figwort family), which contains *Veronica*, has been accomplished. Erdei (1) found maximum axillary shoot production of *Digitalis lantana* in a medium containing 1 mg/liter BA (4.4 μM) and 0.1 mg/liter NAA (0.53 μM). Embryoid production (6, 8) and adventitious bud formation (5, 8, 10) of members of this family also have been studied. The intent of this research was to develop a rapid in vitro multiplication procedure for *V. spicata* 'Red Fox'.

Shoot tips 10 to 15 mm long were removed from actively growing plants maintained in the greenhouse. Leaves were removed, and the shoot tips were disinfested for 10 min with continuous stirring in 0.5% NaOCl (10% Clorox) containing 0.1% Liquinox. Shoot tips then were rinsed in sterile deionized water and trimmed to 5–10 mm lengths. One sterile explant was placed in each culture tube. Stage I medium (3) consisted of Woody Plant Medium (WPM) salts (2), Murashige and Skoog vitamins (4), 30 g/liter sucrose (87.6 μM), 2 mg/liter BA (8.9 μM), 0.02 mg/liter NAA (0.107 μM), and 7 g/liter Difco Bacto-agar. Medium pH was adjusted to 5.7 ± 0.1 and 15 ml dispensed into 25 × 95 mm glass culture tubes; tubes were capped with polypropylene closures (Bellco Kaputs), and autoclaved for 20 min at 121°C. All cultures were maintained at 27° in continuous irradiation at 14 μmol $s^{-1}m^{-2}$ PPF (cool-white fluorescent lamps, F48T12·CW·HO).

Media containing BA, kinetin, or 2iP at 0, 2, 4, 8, 16, 32, or 64 μM were used for shoot multiplication experiments. The cytokinin which produced the greatest number of axillary shoots then was combined with 0.01, 0.1, and 1.0 μM NAA to determine if auxin would affect shoot proliferation. Cultures were completely randomized in the growth chamber and 20 replicates were used in all experiments investigating the effect of cytokinin type on shoot proliferation. Sixteen to 20 replicates were used in the cytokinin and auxin combination experiment. Multiplication experiments were initiated with single shoots (7–10 mm long) obtained from proliferating cultures. Cultures were harvested after 4 weeks and shoot numbers and sizes recorded. Shoots 5 mm long or longer were considered adequate for rooting, but the use of shoots 10 mm long or longer was preferred for easier handling.

The greatest number of shoots 5 mm long and longer was produced in media containing 8 to 16 μM BA at the end of 4 weeks (Table 1). Maximum shoot production in media containing kinetin was 20.4 at 32 μM, whereas a medium containing 64 μM 2iP produced only 14 shoots. For shoots 10 mm long and longer, both 2 and 4 μM BA and 32 μM kinetin produced maximum shoot numbers equal to about 12 shoots per culture (Table 1). Concentrations of cytokinin greater or less than these amounts decreased shoot production when BA and kinetin were considered, whereas 2iP had only a slight stimulatory effect on shoot number at 16 μM.

Since BA was effective at producing shoots in the 5 mm long and longer category, BA at 0, 2, 4, 8, 16, and 32 μM was combined with 0.01, 0.1, and 1.0 μM NAA. The addition of 0.01 or 0.1 μM NAA increased shoots per culture, whereas the addition of 1.0 μM NAA was not promotive, when compared to the media containing BA along (Table 2). Over 30 shoots per culture were produced with the following treatment combinations: 4 μM BA plus 0.01 μM NAA, 8 μM BA plus 0.01 or 0.1 μM NAA, and 16 μM BA plus 0.01 or 0.1 μM NAA. However, the greatest number of shoots, 37.9, was produced on a medium containing 8 μM BA plus 0.01 μM NAA. For shoots 10 mm long and longer, the results were slightly different. Although the addition of 0.01 or 0.1 μM NAA increased shoot number when compared to BA alone, the addition of 1.0 μM NAA showed little difference from BA alone. An average of 14 to 15 shoots per culture developed in media containing 2 μM BA plus 0.01 or 0.1 μM NAA, and 4 μM BA plus 0.01 or 0.1 μM NAA (Table 2). As with media containing BA alone, an optimal concentration or range of concentrations could be determined, above or below which shoot numbers in either size category declined.

Individual shoots 5 mm long or longer were stuck in soilless potting mix [1 sphagnum moss peat: 1 vermiculite (v/v)] in styrofoam trays (19.5 × 13.5 × 7.5 cm) and sealed in plastic bags. After 4 weeks in high humidity, rooted plantlets were given a 2 week acclimation period to ambient humidity by removing the plastic covering gradually before transferring to the greenhouse. Eighty percent of the cuttings rooted. No off type plants have been observed.

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Table 1. Production of shoots by *Veronica spicata* 'Red Fox' in response to 3 cytokinins.

| Concentration (μM) | Mean no. shoots per culture | | | | | |
|---|---|---|---|---|---|---|
| | BA | | Kinetin | | 2iP | |
| | ≥5 mm | ≥10 mm | ≥5 mm | ≥10 mm | ≥5 mm | ≥10 mm |
| 0 | 6.2 ± 0.7[z] | 4.6 ± 0.6 | 5.9 ± 0.7 | 4.6 ± 0.6 | 6.8 ± 0.5 | 5.5 ± 0.5 |
| 2 | 19.0 ± 1.7 | 11.0 ± 1.1 | 6.6 ± 0.7 | 4.8 ± 0.6 | 6.4 ± 0.7 | 5.3 ± 0.5 |
| 4 | 23.2 ± 2.5 | 12.3 ± 1.5 | 7.8 ± 0.9 | 6.2 ± 0.7 | 7.0 ± 0.6 | 5.5 ± 0.5 |
| 8 | 29.8 ± 3.1 | 9.5 ± 1.1 | 8.3 ± 0.6 | 6.6 ± 0.6 | 7.9 ± 1.1 | 5.4 ± 0.8 |
| 16 | 25.8 ± 2.3 | 5.6 ± 0.7 | 9.2 ± 1.1 | 6.9 ± 0.8 | 9.0 ± 0.7 | 7.1 ± 0.6 |
| 32 | 17.1 ± 1.9 | 3.1 ± 0.6 | 20.4 ± 2.9 | 11.8 ± 1.8 | 11.2 ± 1.0 | 6.2 ± 0.7 |
| 64 | 6.4 ± 2.1 | 1.3 ± 0.2 | 13.4 ± 3.1 | 3.9 ± 0.8 | 14.0 ± 1.8 | 4.5 ± 1.0 |

[z]Mean ± SE.

Table 2. Production of shoots by *Veronica spicota* 'Red Fox' in response to BA combined with NAA.

| BA concentration (μM) | NAA concentration (μM)[z] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | | 0.01 | | 0.1 | | 1.0 | |
| | ≥5 mm | ≥10 mm | ≥5 mm | ≥10 mm | ≥5 mm | ≥10 mm | ≥5 mm | ≥10 mm |
| 0 | 6.2 ± 0.7[y] | 4.6 ± 0.6 | 9.0 ± 1.2 | 6.5 ± 0.8 | 5.5 ± 0.5 | 4.1 ± 0.5 | 5.0 ± 0.5 | 4.1 ± 0.4 |
| 2 | 19.0 ± 1.7 | 11.0 ± 1.1 | 21.9 ± 2.1 | 14.8 ± 1.4 | 20.3 ± 1.9 | 14.0 ± 1.5 | 14.1 ± 1.4 | 11.2 ± 1.0 |
| 4 | 23.2 ± 2.5 | 12.3 ± 1.5 | 31.9 ± 2.7 | 14.4 ± 1.5 | 26.1 ± 3.0 | 14.2 ± 1.9 | 22.5 ± 2.1 | 12.7 ± 1.1 |
| 8 | 29.8 ± 3.1 | 9.5 ± 1.1 | 37.9 ± 3.0 | 12.7 ± 1.6 | 34.3 ± 3.0 | 13.8 ± 1.7 | 23.6 ± 1.7 | 10.1 ± 1.0 |
| 16 | 25.8 ± 2.3 | 5.6 ± 0.7 | 35.6 ± 3.5 | 7.9 ± 1.0 | 31.4 ± 2.4 | 10.4 ± 1.3 | 24.7 ± 1.3 | 8.0 ± 0.9 |
| 32 | 17.1 ± 1.9 | 3.1 ± 0.6 | 18.6 ± 2.4 | 4.2 ± 0.8 | 27.4 ± 4.2 | 4.6 ± 1.0 | 24.0 ± 1.9 | 3.0 ± 0.4 |

[y]Mean ± SE, BA values from table 1 added for comparison.
[z]Mean number of shoots per culture.

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# Hardwood Chips as an Alternative Medium for Container Plant Production

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***Abstract.*** **Hardwood chips of post oak, *Quercus stellata* Wangh, and Siberian elm, *Ulmus pumila* L., were used as components of container growth media for *Pyracantha* X 'Mojave' and Formosan sweetgum, *Liquidambar formosana* Hance. Both species grew at least as well in the wood chip media as in conventional pine bark medium. Micronutrients were of little benefit to plants in the oak chip medium but did increase plant growth in the elm chip medium. Drainable pore space decreased dramatically during the growing season, indicating decomposition; however, roots appeared normal when the study ended. Adding additional N above the level generally used with a pine bark medium did not improve growth.**

Pine bark is a major component in many soilless media used in growing container nursery stock. However, prices have increased to the point where alternatives must be considered. Polystyrene beads, rice hulls, rock wool, and crushed shale are examples of materials that have been substituted successfully for pine bark. Nurserymen in the northern United States have been using bark from hardwood trees successfully for the past decade (13).

The chemical property of primary concern when using hardwood materials is the wide C to N ratio. Suggested methods for reducing the wide C:N ratio and to meet the N requirements of plants include composting before use and increasing N fertilization (4, 5, 15, 16, 17).

Gartner et al. (3) substituted hardwood bark for peat and found that various hardwood bark mixes compared favorably to the standard mix of soil, peat, and perlite. Scott and Bearce (11) stated that when properly managed, composted hardwood bark and hardwood sawdust can be used in growth media.

Raker and Hoitink (9) reported composted hardwood bark to be an excellent substrate for production of ericaceous plants. Sterett and Fretz (14) found no significant differences among *Cotoneaster dammeri* 'Royal Beauty' plants grown in a 2 hardwood bark:1 sand (v/v) or a 1 hardwood bark : 1 sand : 1 peat (by volume) medium, regardless of the N source used in the composting process. On the other hand, Smith (12) compared 2 pine bark and vermiculite mixes to several composted hardwood bark and sand mixes where the ratio of bark to sand was varied. Growth of junipers in the pine bark mixes was greater than in any containing hardwood bark.

The pH of hardwood bark media generally is higher than that of mixes containing peat (4, 8, 10). However, the pH of the hardwood bark mix employed by Raker and Hoitink (9) remained nearly the same after 2 growing seasons. Sterrett and Fretz (14) noted the pH of a hardwood bark and sand medium and a hardwood bark, sand, and peat medium increased during the first month of the growing season, followed by a continued decline for

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