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Micropropagation of Virginia Sweetspire (*Itea virginica* 'Henry's Garnet')[1]

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Abstract

The woody shrub, *Itea virginica* L., Virginia sweetspire, has recently increased in popularity due to its multiple seasons of interest in the landscape. In this study, we investigated micropropagation as a means to produce this plant. Combinations of BA (1, 4, and 10 µM) and NAA (0.01, 0.1, and 1.0 µM) were evaluated for in vitro shoot induction in *Itea virginica* L. 'Henry's Garnet' on a Murashige and Skoog medium. The best combination of BA and NAA (4 µM and 0.1 µM) yielded an average of 7.9 microshoots per explant for 'Henry's Garnet'. When dipped in a common auxin-containing, commercial rooting formulation, microshoots rooted ex vitro within four weeks. Tissue-culture produced plantlets of *I. virginica* 'Henry's Garnet' flowered one year after removal from culture.

Index words: tissue culture, in vitro propagation, woody plant.

Species used in this study: Virginia sweetspire (*Itea virginica* L.).

Significance to the Nursery Industry

Virginia sweetspire (*Itea virginica* L.) is a woody shrub native to the eastern United States that has recently become popular for use in the landscape for its attractive white flowers and long-lasting fall foliage color. Several cultivars are available in the nursery trade. Stem cuttings are used to propagate these cultivars. The micropropagation protocol described herein offers another method for the rapid production of new sweetspire cultivars. Plants produced from tissue culture flowered a year after removal from culture.

Introduction

Members of the shrubby genus *Itea* possess desirable horticultural traits of fragrant flowers, attractive fall color, and freedom from major pests and diseases (2, 3, 4). The *Itea virginica* cultivar 'Henry's Garnet' tolerates heavy shade and full sun, wet and dry soils, and is hardy in USDA Zones 5 to 9 (2, 3). Many *I. virginica* cultivars such as 'Henry's Garnet', 'Sprinch' (Little Henry™), and 'Saturnalia' are commonly found in landscapes of the eastern United States. However other deserving cultivars, such as 'Theodore Klein' or novelties like 'Shirley's Compact', are much less well known or used. While the more commonly grown *I. virginica* genotypes are easily propagated by cuttings, a system of micropropagation would be useful to rapidly propagate large numbers of new, deserving cultivars for experimentation and introduction to the nursery industry. Additionally, tissue culture might also be of benefit for propagating useful numbers of the Asiatic, evergreen species of *Itea*. These species (including *I. chinensis* Hook. & Arn., *I. oldhamii* C.K. Schneid., and *I. ilicifolia* Oliv.) are not frequently encountered in the landscape but deserve trial due to their attractive foliage (3).

Materials and Methods

Micropropagation. Actively growing terminal shoot tips (2 cm in length) were harvested from a two-year-old stock plant of *I. virginica* 'Henry's Garnet' maintained in a greenhouse at the University of Arkansas at Fayetteville. Shoot tips were rinsed in running water for 15 minutes, immersed in 70% ethanol (v/v) for 1 minute, and then agitated for 15 minutes in a 100 ml solution of 10% v/v chlorine bleach solution (0.6 % w/v sodium hypochlorite) with 5 drops of Tween-20. After disinfestation, the shoot tips were aseptically trimmed to 1.0 cm and transferred to an initial proliferation medium containing full-strength Murashige and Skoog (7) basal salts, the Linsmaier and Skoog (6) organic supplement (100 mg/l myo-inositol and 0.4 mg/l thiamine), 30 g/l sucrose, 0.5 g/l of the pH buffer MES (2-*N*-morpholinoethanesulfonic acid), 4 µM BA, 0.1 µM NAA, and 6.8 g/l agar adjusted to a pH of 5.7 with 1 M potassium hydroxide. The shoot tip explants consisted of the apex plus one or two nodes and were placed vertically on the medium. Culture vessels were 125-ml glass jars capped with Magenta-B® lids (Magenta Corp., Chicago, IL), and the interface between the lid and the jar was sealed with a single layer of parafilm to prevent excessive moisture loss. The vessels contained 30 ml of the medium. Cultures were maintained at 24C under fluorescent lights (55 µmol $m^{-2}s^{-1}$) for a 16-hr day.

'Henry's Garnet' cultures were maintained on the initial proliferation medium for three 35-day passages until the explants stabilized and consistently produced uniform microshoot propagules. Following these initial passages, sufficient uniform microshoots were produced to initiate a shoot proliferation study. The basal proliferation medium was the same composition as the initial proliferation medium, supplemented with all combinations of 1 µM, 4 µM, and 10 µM of BA and 0.01 µM, 0.1 µM, and 1.0 µM of NAA. The environmental conditions were the same as previously mentioned. One additional treatment containing neither BA nor NAA served as a reference to determine if plant growth regulators produced an effect on proliferation. The experimental unit consisted of a culture vessel containing a single 0.5-cm terminal microshoot (consisting of the apex and one or two nodes). Each treatment was replicated five times, and the experiment was repeated twice for a total of three subcultures with the data collection occurring after the experimental units had grown on the treatment media for 35 days.

Following each of the three 35-day passages, the number of new microshoots induced by each treatment was assessed

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by dividing the resultant microshoot clumps and counting the number of new, usable microshoots (≥ 0.5 cm in length) per replicate. Since only new microshoots were counted, the original explants were excluded from all treatment counts. The explants on the treatment lacking plant growth regulators elongated and produced roots *in vitro* but were excluded from the statistical analysis because they did not produce new microshoots.

For ex vitro rooting and acclimation, microshoots (≥ 0.5 cm in length) of *I. virginica* 'Henry's Garnet' were dissected from the microshoot clumps and dipped in Rootone F® — a rooting powder composed of 0.2% 1-naphthaleneacetamide and 4.04% tetramethylthiuramdisulfide (Green Light®, San Antonio, TX). The microcuttings were placed vertically into Bio sponge plugs (Park's Seed Company, Greenville, SC) which were then enclosed in the Park's Bio Dome seed starter box. After 14 days the vents on the box were opened, and following an additional 14 days (when roots from the microcuttings were observed to have spread throughout the plugs) the plantlets were transferred to pots and placed in a greenhouse.

Statistical analysis. The experiment was planned as a completely randomized design with a 3 × 3 factorial arrangement of treatment levels. Microshoot data were analyzed with the PROC ANOVA procedure of SAS (SAS Institute Inc., Cary, NC). The main effects analyzed were BA treatment effects, NAA treatment effects, and time effects as delineated by passage (passage one represented the microshoot data collected after the first 35-days, passage two represented the microshoot data collected after the experiment was repeated and maintained for another 35-days, and so on), and the interactions between the main effects were analyzed for significance. Proc RSREG (SAS Institute Inc., Cary, NC) was used to test for regression effects.

Results and Discussion

The BA and NAA treatment main effects were significant while the passage main effect was not significant (Table 1). Passage × BA, passage × NAA, and BA × NAA interactions were not significant (Table 1). The lack of significance for the passage main effect indicates that the proliferation of 'Henry's Garnet' explants did not decline or increase (outside of treatment effects) from one 35-day passage to another illustrating that the selected plant growth regulator treatments consistently affected proliferation over time. Furthermore, this consistent response over time implies that the preliminary three initiation passages prior to the introduction of plant growth regulator treatments were sufficient for stabilizing the explant growth of 'Henry's Garnet' (1, 5).

Average microshoot production per explant was higher with 4.0 µM BA (n = 45) than the other cytokinin levels, and microshoot production was higher with 0.1 µM NAA (n = 45) than with the other auxin levels (Table 2). For both growth regulators, moderate concentrations, compared to low and high concentrations, resulted in greater microshoot means, indicated by a significant quadratic trend (Table 2). The treatment combining 4 mM BA with 0.1 mM NAA yielded an average of 7.9 (n = 15) new microshoots per explant for 'Henry's Garnet'. No other efforts in the micropropagation of Iteaceae have yet been reported; however, the average microshoot proliferation achieved here for 'Henry's Garnet' compares favorably with the maxium proliferation rate of 3.53 (n = 15) microshoots per experimental unit achieved by Brennan et al. (1) on the *Ribes nigrum* L. (black currant) cultivar 'Ben Lomond' with a Murashige and Skoog (7) medium supplemented with 9.8 µM (2.2 mg/l) BA. Black currant is a member of the Grossulariaceae family, a family closely related to Iteaceae (1,3). The differences between the average proliferation rates for 'Henry's Garnet' and 'Ben Lomond' can most likely be attributed to genetics and the duration of the passages — 35 days for the former and 21 days for the latter (1).

Ex vitro rooting of microshoots occurred within four weeks after the rooting treatment. Rooted and acclimated plants of *I. virginica* 'Henry's Garnet' that were planted in the field normally flowered one year after removal from culture. We have used this protocol to successfully produce the *I. virginica* cultivars 'Saturnalia', 'Sarah Eve', 'Long Spire', 'Sprinch' (Little Henry™), 'Merlot', and 'Theodore Klein' as well as the evergreen species *I. ilicifolia*, *I. oldhamii*, *I. chinensis*, and *I. yunnanensis* Franch. In vitro proliferation of these taxa (data not shown) was favorably comparable (or better) to the proliferation reported here for 'Henry's Garnet'. The information generated by this research may be applicable to the rapid propagation of other valuable *Itea* genotypes for dissemination throughout the nursery industry.

Table 1. Analysis of variance summary table for *Itea virginica* 'Henry's Garnet' microshoot proliferation data.

Source of Variation	df	MS	F	P > F
BA	2	45.74	7.12	0.0012
NAA	2	124.63	19.39	<0.0001
Passage	2	7.34	1.14	0.3229
BA × NAA	4	14.76	2.30	0.0636
Passage × BA	4	10.74	1.67	0.1619
Passage × NAA	4	7.10	1.10	0.3584
Passage × BA × NAA	8	2.13	0.33	0.9522
Model	26	19.34	3.01	<0.0001
Error	108	6.43	—	—
Corrected total	134	—	—	—

Table 2. Average microshoot number produced per *Itea virginica* 'Henry's Garnet' explant.

Level µM	Microshoot means[z]
BA	
1.0	3.20
4.0	5.20
10.0	3.98
Significance	Quadratic[y]
NAA	
0.01	2.76
0.1	5.98
1.0	3.64
Significance	Quadratic[y]

[z]Values are means of five replications with data being collected for the treatments at the end of three 35-day passages (n = 45).

[y]significant at $P \leq 0.001$.

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