POSTER SESSION

In Vitro Regeneration of *Cladrastis kentukea*©

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Axillary buds from a single *Cladrastis kentukea* tree were cultured initially on two media, Woody Plant Medium (WPM) and Murashige and Skoog (MS), containing 0, 1, 2, or 4 μM 6–benzylaminopurine (BA). Cultures were transferred to fresh media every 4 weeks. Elongated shoots were harvested after 39 weeks and transferred to half-strength MS medium supplemented with following concentrations of IBA: 0, 3, 30, 100, and 300 μM. The most (75%) explants rooted when exposed to the highest concentrations of IBA. Although this treatment yielded the most rooted plantlets, there was significantly higher terminal meristem abortion compared to other treatments. There were no statistical differences between the numbers of roots and total root length among all treatments.

INTRODUCTION

Cladrastis kentukea (Dum.-Cours.) Rudd (American yellowwood) is an ornamental tree and a member of the Fabaceae. The genus *Cladrastis* encompasses only four species, of which three are native to southeastern Asia (Harrar, 1971). Yellowwood, described in 1812, is native to North Carolina, Kentucky, and Tennessee, but is not very common (Dirr, 1998). Yellowwood is difficult to propagate by conventional vegetative methods; nevertheless, commercial propagation of yellowwood relies on seed, which requires scarification and stratification (Dirr, 1998). Seed supply may be limited from year to year due to yellowwood's sporadic blooming cycles. Yellowwood has ornamental qualities and the potential for wider geographical distribution and availability. However, its value in nursery and landscape production can be limited by lack of an efficient method of propagation (Weaver, 1990).

A major advantage of micropropagation in angiosperm trees is the wide range of explants available such as seeds, seedling parts, leaves, shoots, buds from both mature and juvenile trees, floral parts, and in some cases even roots (Khurana et al., 2003). Propagation by means of tissue culture can enhance plant efficiency and provide year-round production with high quality plants, offering rapid mass propagation methods, genetic uniformity of a clone as well as an alternative technique of propagation for this native species. Although axillary-bud proliferation and somatic embryogenesis have been accomplished in the Fabacae, requirements for in vitro propagation of legumes are species dependent (Weaver, 1990). The only tissue culture propagation method for yellowwood was reported by Weaver and Trigiano (1991).

The objective of this study was to develop a tissue culture system for clonal propagation of yellowwood and provide faster and more efficient methods for its commercial propagation.

MATERIALS AND METHODS

All yellowwood plant material originated from a single tree on the University of Tennessee Agricultural campus in Knoxville, TN, eliminating genotype effects. Axillary buds were removed from elongated branchlets during the last week of April before anthesis. After the lamina and most of the petiole were eliminated, nodal segments were removed one centimeter above and below enlarged clasping petiole bases enclosing the axillary buds. Stem segments were dipped in 95% ethanol, flamed for 10 sec, and then surface sterilized for 8 to 10 min in 20% v/v commercial bleach solution (Clorox®) amended with 0.1%Triton X-100 with constant stirring. Explants were rinsed three times with sterile distilled water. After surface sterilization procedures, the cut ends exposed to the bleach were removed, and axillary buds placed in 20 × 150-mm glass culture tubes. The two basal media, Murashige and Skoog (MS) (Murashige and Skoog, 1962) and Woody Plant Medium (WPM) (Lloyd and McCown, 1980), were amended with 0, 1, 2, or 4 μM 6–benzylaminopurine (BA). In the previous study (Weaver, 1990), yellowwood axillary buds produced the most consistent number of shoots and performed the best overall, which was evident in this research as well, in culture supplemented with either 2.2 or 4.4 μM BA. After 20 weeks, all explants were placed in tissue culture jars, regardless of initial BA concentration, on medium containing 2 μM BA. Cultures were transferred every 4 weeks to fresh medium. They were maintained in incubators at 23 ºC under 25 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ light intensity for 16/8 light-dark photoperiod. Over the time, WPM medium was unsuitable for yellowwood in vitro production, resulting in stunted growth, necrosis, yellowing, and overall poor performance of the plants, and was excluded from the study.

Thirty-nine weeks after initial culture, elongated shoots were harvested and transferred to Magenta GA-7 vessels containing half-strength MS medium supplemented with $6 g \cdot L^{-1}$ of agar. Indole-3-butyric acid (IBA) was filter-sterilized using a 0.22 μm syringe filter and added after the medium had cooled but not hardened at the following concentrations: 0, 3, 30, 100, and 300 μM. After an initial pulse treatment of 3 days, explants were placed in 17 × 100 mm plastic, disposable culture tubes with plastic snap-on caps, containing half-strength MS without plant growth regulators. The experimental design was a randomized complete block design with four explants per each Magenta vessel and six replications for each treatment. The experimental unit consisted of one microshoot per culture tube, resulting in 120 explants. Proliferating masses were transferred to fresh medium with 2 μM BA and were used again for the rooting projects. Data was analyzed using analysis of variance (ANOVA) and means separated using LSD t test (alpha=0.05) (SAS Institute, 1996).

RESULTS AND DISCUSSION

After 4 weeks, data was collected including the number of rooted microshoots, number of roots per rooted microshoot, length of each root, number of damaged meristems, presence or absence of lenticels, and number of roots.

More microshoots exposed to the highest concentrations of IBA rooted than did at lower concentrations of IBA. In total, 18 out of 24 explants rooted when exposed to 300 μM IBA for 3 days, resulting in 75% rooted microshoots (Table 1). Fifty-four percent of the microshoots exposed to 100 μM IBA rooted, representing a total of 13 rooted plants. There was no significant difference between the numbers of mi-

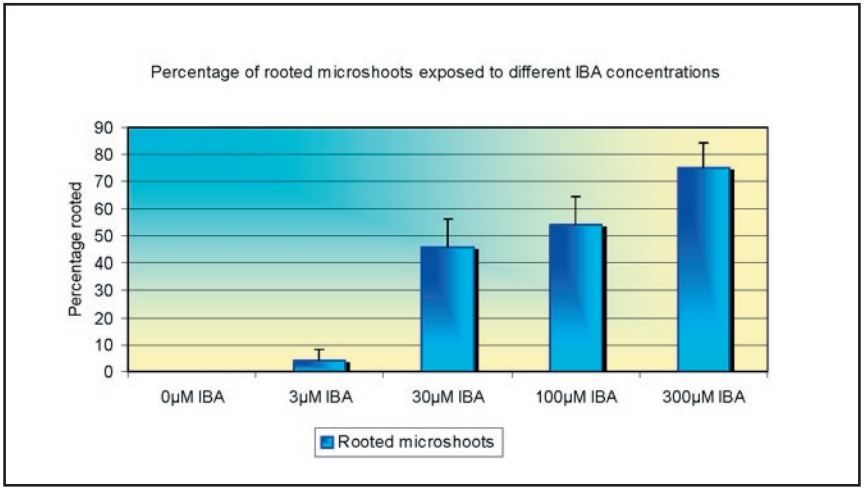

Figure 1. Effect of IBA on root formation of *Cladrastis kentukea* microshoots. Vertical bars represent standard error.

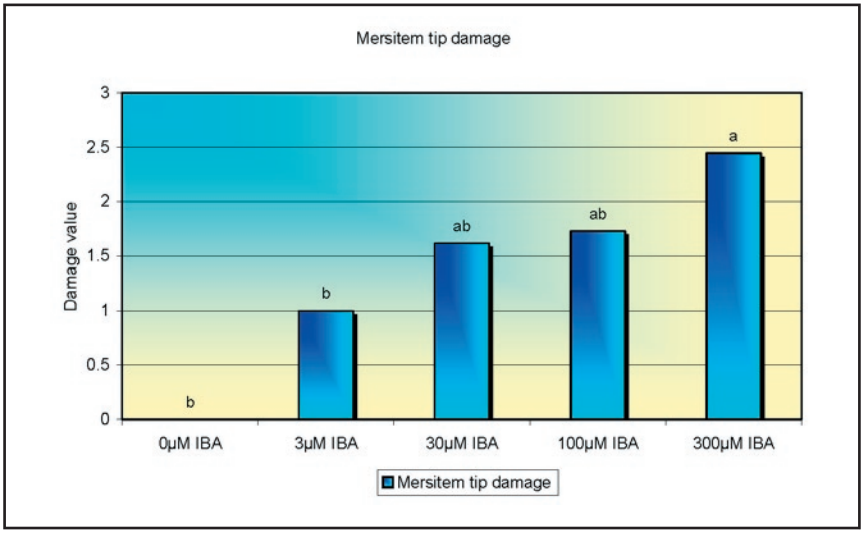

Figure 2. Effect of IBA on meristem tip damage of *Cladrastis kentukea* microshoots. Statistical differences were assessed by LSD t means separation test.

Table 1. Effect on rooting of *Cladrastis kentukea* microshoots after 4 weeks on Murashige and Skoog medium supplemented with IBA. Values within a column with different letters are significantly different ($P< 0.05$).

IBA conc. (μM)	Number of rooted explants/ total number of explants	Mean percentage of rooted explants	Standard error	Mean number of roots/rooted microshoot	Mean number of total root length/ rooted microshoots (mm)	Mean shoot damage/ rooted microshoot
0	0/24	0 c	0	0 a	0 a	0 b
3	1/24	4.1 c	0.04	5 a	122.4 a	1 b
30	11/24	45.8 b	0.10	4.45 a	108.05 a	1.72 ab
100	13/24	54.1 b	0.10	4.46 a	96.39 a	1.61 ab
300	18/24	75.0 a	0.09	6.61 a	104.11 a	2.44 a

croshoots exposed to 30 and 100 μM IBA treatments. Only 4% of the microshoots rooted when exposed to 3 μM IBA for three days, representing a single plant, and none of the control (without IBA) microshoots rooted (Table 1). There were no statistical differences between the numbers of roots and total root length among all treatments (Table 1). However, all microshoots that rooted had lenticels, suggesting that presence of lenticel cambial activity can possibly improve rooting abilities of selected microshoots.

Significant differences (*P<0.05)* were observed with the explants exposed to 300 μM of IBA compared to other treatments (Fig.1). Although this treatment induced the most root formation, there was significantly higher ($P<0.025$; $F=4.11$) terminal meristem abortion compared only to the microshoots treated with 100 and 30 μM IBA (Fig. 2). This suggests that better balance between exposure and timing of auxin treatments for rooting is critical and will require further studies. When exposed to concentrations of 100 and 30 μM of IBA, the number of microshoots that rooted did not significantly differ — 54% and 45% of microshoots rooted, respectively. Also, when comparing meristem tip damage, these two treatments showed no significant difference between each other ($P=0.7756$; $F=0.08$), however, there was significantly less damage ($P<0.0118$; $F=7.06$ and 0.0356; $F=4.78$, respectively) than with microshoots rooted on 300 μM (Fig. 2).

Figure 3. A cloned and acclimatized plant of *Cladrastis kentukea.*

Rooted microshoots were washed with tap water to remove agar and placed in 5.5-cm plastic pots containing autoclaved, general-purpose medium — Promix BX (Premier Horticulture Ltd, Pennsylvania). Pots with rooted microshoots were placed in 323 × 228 × 46 mm Hefty cake aluminum pans with plastic lids (Pectiv Corporation, Lake Forest, Illinois) and gradually acclimatized to nonsterile environment (Fig. 3).

CONCLUSIONS

Selection of superior individuals followed by clonal vegetative propagation is a very important strategy for plant improvement. Cloning via tissue culture can produce a population of the same identical genotype without limits. Efficient tissue culture systems can enhance yellowwood propagation methods without relying on conventional techniques; however, these systems yield limited success and are seasonally dependent. Faster, more efficient commercial propagation methods, for yellowwood with superior phenotypes, offer continuous, year-round production of high quality plants to growers. This study presented protocols and established methods for *C. kentukea* in vitro regeneration, providing new approach to mass production of this valuable ornamental tree for the southeast region of the United States.

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