HRI's Mission:

To direct, fund, promote and communicate horticultural research, which increases the quality and value of ornamental plants, improves the productivity and profitability of the nursery and landscape industry, and protects and enhances the environment.

Micropropagation of White Flowering Eastern Redbud (*Cercis canadensis* var. *alba* L.)[1]

S.Yusnita, R. L. Geneve and S. T. Kester[2]
Department of Horticulture and Landscape Architecture
University of Kentucky, Lexington, KY 40546

Abstract

A white flowering Eastern redbud (*Cercis canadensis* var. *alba* L.) has been successfully micropropagated. Two node explants collected from the initial flush of spring growth were cultured on woody plant medium (WPM). Increased shoot multiplication occurred at 10,15 and 20 μM (2.3, 3.4 and 4.5 ppm) benzyladenine (BA). Microshoots were rooted *in vitro* on half strength WPM with a 15-day treatment of 100 and 300 μM (18.6 and 55.9 ppm) α-naphthaleneacetic acid (NAA) or 100 and 300 μM (20.3 and 60.9 ppm) indolebutyric acid (IBA) prior to being moved to full strength WPM without growth regulators. Percentage rooting and the mean number of roots per cutting were comparable between NAA and IBA treated microcuttings, however, the subsequent root morphology differed between the two treatments. NAA treated plants developed a coarse, unbranched root system, while IBA treated cuttings developed a more desireable fine, branched root system. Rooted microshoots were successfully acclimated to greenhouse conditions.

Index words: tissue culture, root formation

Significance to the Nursery Industry

Micropropagation offers a viable alternative to T-budding for the propagation of selected cultivars of Eastern redbud. The present study demonstrates that mature forms of Eastern redbud can be successfully micropropagated. Commercial tissue labs are interested in propagating Eastern redbud, but root formation on developed microshoots remains a problem. This study demonstrates that cuttings treated with NAA or IBA for 15 days *in vitro* rooted readily. However, the defoliation of some cuttings and the terminal necrosis observed in stock cultures indicates that further work is necessary before the micropropagation of mature forms of Eastern redbud becomes a routine mass propagation method.

Introduction

Eastern redbud (*Cercis canadensis* L.) is a small to medium-sized tree native to the North Eastern United States. It is a popular landscape tree displaying bright lavender, pink or white flowers in the early spring before the foliage appears.

The nursery industry commonly propagates redbud from seed. Several cultivars are available in the trade, but these have proven difficult-to-root from cuttings and are propagated with variable success by T-budding. Recently, Tipton (6) reported success in rooting softwood cuttings from a seven-year-old Mexican redbud (*C. canadensis* var. *mexicana*), but only during a limited time after the spring flush of growth.

A rapid micropropagation system for Eastern redbud would benefit the nursery industry by increasing the availability of selected cultivars like the purple-leaved 'Forest Pansy' and the white flowering form of Eastern redbud (*C. canadensis* var. *alba*). Bennet (1) reported the successful micropropagation of Mexican redbud. However, this was apparently performed with greenhouse-grown seedling material. It was not reported, but Bennett implied microshoot production in the cultivars 'Forest Pansy' and 'Oklahoma'. In this paper, we report the successful micropropagation of white flowering Eastern redbud from a mature landscape specimen.

Material and Methods

Explants were obtained from rapidly growing shoots during the initial flush of vegetative growth in May, 1988. Shoots were collected from a single tree of white flowering Eastern redbud (approximately 15-years-old) located on the University of Kentucky campus. Leafless explants, 3 cm (1.2 in) in length were washed in running tap water for 1 hour. This was followed by sequentially treating the explants with 70% ethanol, 1500 ppm benomyl and a solution of 0.5% sodium hypochlorite (10% Chlorox) plus 0.1% Alconox detergent for 10 sec, 10 min and 15 min, resp. The disinfestants were removed by three rinses with autoclaved, deionized water.

Approximately 50% of explants were successfully disinfested following this procedure. Ten explants were subcultured every 7 weeks on woody plant medium (WPM) (4) supplemented with 10 μM (2.3 ppm) benzyladenine (BA). Explants were cultured in Magenta jars (Carolina Biological Co. NC) containing 50 ml (1.7 oz) medium and placed under a 16 hr photoperiod provided at 30 $\mu mol \cdot sec \cdot^{-1} \cdot m^{-2}$ of light by cool white fluorescent lamps. Temperature was maintained at 24°C (75°F). After eight subcultures, a sufficient number of explants were available to initiate dose response studies of cytokinins.

Dose response studies were conducted using BA and thidiazuron (TDZ) on 2 node explants maintained under the cultural conditions described previously. The concentration levels of BA were 5, 10, 15, and 20 μM (1.1, 2.3, 3.4 and 4.5 ppm). TDZ was tested at 0.05, 0.5, 5, 10, 15, and 20 μM (0.1, 1.1, 2.2, 3.3 and 4.4 ppm). Microshoots were harvested and the original explants were subcultured to the same concentration of cytokinin medium every 7 weeks. Each treatment consisted of 20 explants (4 explants per jar)

[1]Received for publication April 16, 1990; in revised form June 4, 1990. Published as Kentucky Agriculture Experiment Station Jounral Article *90-10-57*.

[2]Graduate student, assistant professor and lab technician, resp.

and the jars were arranged in a completely randomized design. The experiment was repeated three times.

In vitro rooting experiments were carried out with axillary microshoots ≥ 3 cm (1.2 in) long from explants treated with 10 μM (2.3 ppm) BA. Microshoots were treated with a 5 sec quick dip (50% ETOH) of indole-3-butyric acid (IBA) or α-naphthaleneacetic acid (NAA) at 50, 100, and 150 μM (10.2, 20.3, 30.5, and 9.3, 18.6, 27.9 ppm, respectively) prior to being moved to full strength WPM without growth regulators for root development. Microshoots were also treated for 15 days on half strength WPM containing IBA or NAA at 100 or 300 μM (20.3, 60.9 and 18.6, 55.9 ppm, respectively) before being moved to full strength basal WPM. Rooting percentage and number of roots per cutting were recorded after 4 weeks in culture. Each treatment consisted of 15 cuttings in a completely randomized design and the experiment was repeated twice. Rooted cuttings were acclimated by potting them in a greenhouse soil mix and gradually decreasing the humidity under intermittent mist.

Results and Discussion

BA was effective in promoting axillary bud break and shoot development (Table 1). There was a significant increase in shoot multiplication with each subculture and the greatest number of shoots was obtained after three subcultures on a medium containing 20 μM (4.5 ppm) BA. After three subcultures, there was no statistical difference for shoot multiplication between cultures treated with 10, 15 or 20 μM (2.3, 3.4, 4.5 ppm) BA. Stock cultures are currently maintained on 10 μM (2.3 ppm) BA, where there is consistently good shoot multiplication from predominantly axillary shoots. After three subcultures, many of the developing shoots showed terminal necrosis that required cultures to be re-initiated from two-node explants obtained from stock cultures. Terminal necrosis has been observed in several woody species in culture including chestnut (7), birch, apple, redwood, elm and rhododendron (5). For potato tissue cultures, terminal necrosis has been overcome by increased calcium in the medium (5). The cause of terminal necrosis in redbud cultures has not been evaluated.

In contrast to BA-treated cultures, TDZ, at the concentrations tested, was ineffective for promoting axillary shoots that could be used for micropropagation. Typcially, TDZ treated explants were characterized by numerous axillary and adventitious shoot production. However, these shoots failed to elongate and many of the shoots were distorted or fasciated (Data not shown).

Adventitious root formation in stem cuttings has been reported to be difficult for Eastern redbud (2), although a recent report indicated that softwood cuttings from Mexican redbud could be rooted using a high humidity fog system for cuttings treated with a high concentration of IBA (6). Microshoots of white Eastern redbud rooted easily after being treated for 15 days on a medium containing 300 μM

Table 1. Shoot multiplication in white Eastern redbud treated with various concentrations of BA.

BA Concentration		Subcultures		
		1st	2nd	3rd
[μM]	[ppm]	Number of shoots per cutting		
5	1.1	1.6 ± 0.3[z]	2.5 ± 0.5	3.2 ± 0.6
10	2.3	2.3 ± 0.4	3.5 ± 0.4	4.8 ± 0.8
15	3.4	2.6 ± 0.4	4.3 ± 0.6	5.5 ± 0.9
20	4.5	2.0 ± 0.3	4.7 ± 0.6	5.9 ± 1.0

ANOVA	F value	Significance[y]
Treatment	26.0	**
BA Conc.	83.5	**
Subculture	6.2	**
BA X Subculture	5.7	*

[z]Mean number of shoots per explant ± 95% confidence interval.
[y]**,*is significant at .01 and .05 level, resp.

Table 2. The effect of 15 day pulse treatment of IBA and NAA on rooting percentage and mean number roots per cutting in white Eastern redbud.

Auxin Concentration		Number of explants rooted	Number of roots per rooted cutting
[μM]	[ppm]	(%)	
IBA			
100	20.3	53	8.1 ± 3.95[z]
300	60.9	73	18.7 ± 6.42
NAA			
100	18.6	60	10.8 ± 2.78
300	55.9	93	9.5 ± 4.61
Control		20	1.3 ± 0.65

[z]Mean number of roots ± 95% confidence interval.

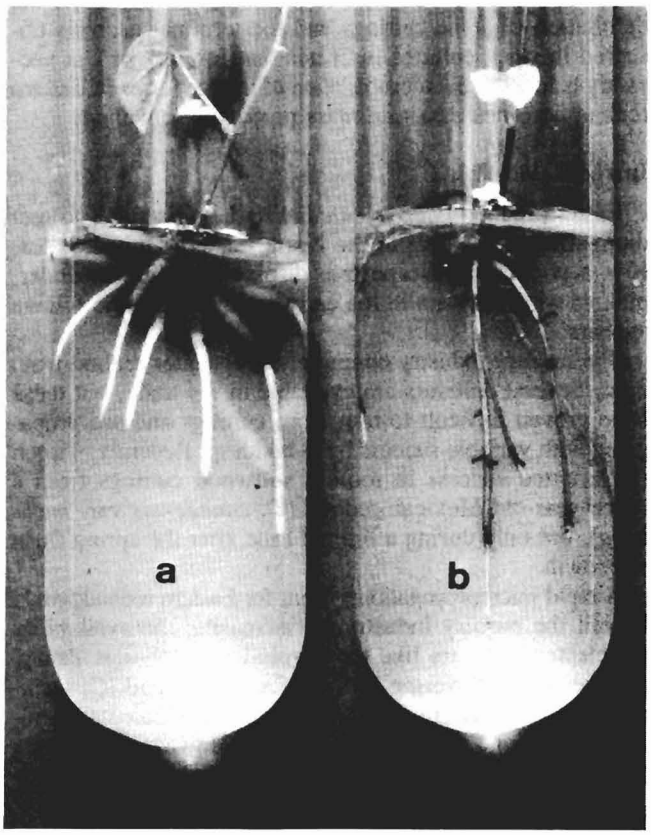

Fig. 1. Root morphology in microshoots of Eastern redbud treated with (a) 300 μM (55.9 ppm) NAA or (b) 300 μM (60.9 ppm) IBA.

(55.9 ppm) NAA or 300 μM (60.9 ppm) IBA prior to being moved to a medium without growth regulators (Table 2). Rooting percentages between 73 and 93% were obtained with these treatments *in vitro*. There was no root formation observed in cuttings treated with the quick dip method for either NAA or IBA (Data not shown).

NAA and IBA were both effective in initiating root formation. However, the roots formed in NAA-treated cuttings were short and thick with no secondary branching. IBA treated cuttings produced a finer, more branched root system than NAA treated cuttings (Fig. 1).

Microshoots developed from a mature form of Eastern white redbud have a high potential for root formation. This high rooting potential was probably related to the number of subcultures for these explants prior to the rooting experiments. Successive subculturing has been related to increased rooting potential in a number of species (3). Rooted microshoots were acclimated to greenhouse conditions, but these have not flowered to demonstrate maintenance of the white flowering phenotype.

Literature Cited

1. Bennett, L. 1987. Tissue culturing redbud. American Nurseryman. 166(7):85–87, 90–91.

2. Dirr, M.A. and C.W. Heuser. 1987. The Reference Manual of Woody Plant Propagation. Varsity Press, Athens, GA 30603.

3. Hackett, W.P. 1988. Donor plant maturation and adventitious root initiation, p. 11–28. *In*: (eds.) T.D. Davis, B.E. Haissig, and N. Sankhla, Adventitious Root Formation in Cuttings. Dioscorides Press, Portland, Oregon.

4. Lloyd, G. and B. McCown. 1980. Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. Proc. Intern. Plant Prop. Soc. 30:421–427.

5. Sha, L., B.H. McCown, and L.A. Peterson. 1985. Occurrence and cause of shoot-tip necrosis in shoot cultures. J. Amer. Soc. Hort. Sci. 110:631–634.

6. Tipton, J.L. 1980. Vegetative propagation of Mexican redbud, larchleaf goldenweed, littleleaf ash, and evergreen sumac. HortScience 25:198–199.

7. Vietez, A.M., C. Sanchez, and C. San-Jose. 1989. Prevention of shoot-tip necrosis in shoot cultures of chestnut and oak. Scientia Hort. 41:151–159.

Control of Basal Sprout Regrowth on Crapemyrtle with NAA[1]

Gary J. Keever[2] and William J. Foster[3]
Department of Horticulture
Alabama Agricultural Experiment Station
Auburn University, AL 36849

Abstract

Inhibition of basal sprout development on *Lagerstroemia indica* L. × *fauriei* Koehne 'Natchez' and *L. indica* L.'Country Red' was achieved with 1-naphthaleneacetic acid (NAA) applied as a directed trunk spray or in a lanolin paste. A directed trunk spray was more effective than lanolin paste application in reducing sprout number and dry weight; plant height of 'Country Red', but not 'Natchez' crapemyrtle, was reduced by spray application compared to lanolin paste application or the control. Sprout number and dry weight of both crapemyrtle cultivars, shoot dry weight of both cultivars sprayed, and plant height of sprayed 'Country Red' crapemyrtle decreased with increasing NAA rate. NAA rate did not affect shoot dry weight of either cultivar or height of 'Country Red' crapemyrtle treated with NAA in a lanolin paste. Height of 'Natchez' crapemyrtle treated by either method of application was not affected by NAA rate.

Index words: auxin, naphthaleneacetic acid, growth regulator

Growth regulator used in this study: NAA (1-naphthaleneacetic acid).

Species used in this study: 'Natchez' crapemyrtle (*Lagerstroemia indica* L. × *fauriei* Koehne 'Natchez'); 'Country Red' crapemyrtle (*L. indica* L. 'Country Red').

Significance to the Nursery Industry

Considerable expense is incurred when hand pruning basal sprouts from tree-form crapemyrtles. Application of NAA either as a directed trunk spray or in lanolin paste suppressed development of basal sprouts on crapemyrtle. Greater control of basal sprouts occurred with directed sprays as the concentration of NAA increased from 0 to 1%. An average of 0.4 sprouts developed on plants sprayed with 0.75% NAA, while no sprouts formed on plants sprayed with 1.0% solution. Directed sprays suppressed overall shoot growth when compared to the control. Due to the time required to paint pruning cuts, NAA applied in a lanolin paste is not a viable option for nurserymen.

[1]Received for publication March 29, 1990; in revised form June 11, 1990. Alabama Agricultural Experiment Station Journal No. 11-902504P.

[2]Associate Professor of Horticulture.

[3]Former Superintendent, Ornamental Horticulture Substation, P.O. Box 8276, Mobile, AL 36689.

Introduction

Crapemyrtles are popular landscape plants in the Southeastern U.S. Colorful blooms during the summer months,