TISSUE CULTURE PROPAGATION OF *SOPHORA SECUNDIFLORA*

CAL A. FROBERG

Department of Horticultural Sciences
Texas A&M University
College Station, Texas 77843

Abstract. *Sophora secundiflora* can be established *in vitro* by sterilizing explant material in 1% Liquinox for 3 min., 70% ETOH for 3 min., 10% Clorox for 30 min., with 3 rinses in sterile water. Explants can be multiplied on WPM with 5, 7.5, or 10 mg/l BA. There is currently not an adequate treatment for shoot elongation. Plantlets can be rooted using a quick-dip for 5 min. in 1000 ppm IBA.

Sophora secundiflora, Texas mountain laurel, is a woody-ornamental shrub native to west Texas that has considerable value in the landscape. Some of the desirable characteristics include dark evergreen foliage, fragrant blue flowers, as well as drought and pest resistance. Currently, *Sophora secundiflora* is commercially propagated by seed, since cuttings have very low rooting ability. Because of the genetic variability in seedlings, clonal propagation is desirable which will permit selection for specific characteristics such as flower color, growth habit, and drought tolerance. Within the past several years tissue culture propagation has been used to propagate many woody plants (2). Woody ornamentals such as sweetgum (8), red maple (9), oak (3), and birch (6) have been successfully micropropagated. In order to clonally propagate Texas mountain laurel, it is necessary to use tissue culture techniques since other asexual methods have not been commercially feasible. The objectives of this study were:

1. To determine the best sterlization procedure to establish explants *in vitro*.
2. To determine the best medium and growth regulator(s) concentration to induce multiplication.
3. To determine the best growth regulator and concentration to induce rooting.
4. To acclimatize the plantlets successfully.

MATERIALS AND METHODS

Plant material for *in vitro* propagation was obtained from one-year-old greenhouse-grown seedlings. All seeds were collected from a single tree located on the Texas A&M campus. Plants were grown under 18-hr. daylength in order to prevent dormancy. Single-node stem sections 1 to 2 in. (2.5 to 5.0 cm.) in length as well as shoot tips were used as explants.

Three different sterilization procedures were tested. They were:

1). 3 min. 1% Liquinox, 3 min. 70 ethanol, 15 min. 10% Clorox, rinsed 3 times with sterile distilled water plus 2% Clorox.

2). 3 min. 1% Liquinox, 3 min. 70% ethanol, 30 min. 10% Clorox, rinsed 3 times with sterile distilled water plus 2% Clorox.

3). 3 min. 1% Liquinox, 3 min. 70 ethanol, 30 min. 0.01% $KMnO_4$ (1), rinsed 3 times with sterile distilled water plus 2% Clorox.

All leaves were removed prior to sterilization and stems were cut into 3 to 4-inch (7.6 to 10.2 cm.) sections.

Explants were cultured on Woody Plant Medium (WPM) (5) with 0.8% Bacto-Difco agar (8 g/l). Multiplication medium contained either no growth regulators, or benzyladenine (BA) at 1, 3, 5, 7.5, and 10 mg/l, or 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine (PBA) (4) at concentrations of 1, 3, and 10 mg/l. Elongation medium contained either no growth regulators or gibberellic acid (GA_3) (10) at concentrations of 0.1, 0.3, 1, 3, and 10 mg/l.

All media were adjusted to pH 5.7 before adding agar; 10 ml was placed in each 150 × 25 mm test tube. The test tubes were capped with Magenta caps and autoclaved at 121° C (247° F) for 15 min. Tubes were cooled upright. Cultures were placed on a growth shelf under cool white fluorescent lights on a 16-hr. photoperiod. The temperature was kept at 25° ± 1°C (77° ± 1°F). Cultures were transferred every three to four weeks. For rooting, shoots were excised and quick-dipped in indolebutryic acid potassium salt (K-IBA) for 5 min. at concentrations of 500 and 1000 mg/l. They were then stuck in moist peat pellets that were soaked in half strength WPM (5) with the pH adjusted to 5.7 Pellets with explants were kept under clear plastic cups on the growth shelf. Holes were punched in the cups to reduce humidity and acclimatize the plantlets.

RESULTS

Sterilization. Of the three sterilization procedures tested, the 30 min. treatment with 10% Clorox had the highest percent of clean cultures (Table 1). Most of the contamination was from bacteria — very little was fungi, but the percentage of clean cultures was lower than desired. To improve this, stock plants were drenched every 3 to 4 wks. with Subdue at 1 oz./100 gal. of water or Banrot at 8 oz./100 gal. of water. Handling techniques during sterilization were also adjusted. These changes led to an increase in clean cultures from 28% to 40%.

Table 1. Sterilization results of *in vitro*-propagated *Sophora secundiflora*. All sterilization treatments included an initial 3 min. 1% Liquinox and 3 min. 70% ethanol application[1].

Treatment	Percent clean cultures
15 min. 10% Clorox	11
30 min. 10% Clorox	28
30 min. 0.01% $KMnO_4$	12

[1] 36 explants per treatment.

Multiplication. Multiple shoots were formed on media containing cytokinins. BA at 5, 7.5 and 10 mg/l was more effective than PBA in promoting bud break and shoot development (Table 2). BA at 7.5 mg/l produced 5.3 shoots per explant.

Table 2. Effect of BA and PBA on shoot multiplication of micropropagated *Sophora secundiflora*[1].

Treatment	Avg. number of shoots/explant
Control	1.0
BA 1 mg/l	1.5
BA 3	2.2
BA 5	4.4
BA 7.5	5.3
BA 10	5.0
PBA 1	1.4
PBA 3	2.0
PBA 10	2.5

[1] 15 explants per treatment

Elongation. Since a very small percentage of shoots sufficiently elongated for easy handling, explants were transferred to media containing either no growth regulator or GA_3 (9). The percentage of shoots greater than 5 mm was not significantly different among any of the treatments including the control (Table 3). The percentage of long shoots was not, however, as high as desired.

Table 3. Effect of GA_3 on alongation of *Sophora secundiflora* shoots propagated *in vitro*[1].

Treatment	Avg. number of shoots>5mm
GA 0 mg/l	0.3
GA 0.1	0.4
GA 0.3	0.2
GA 1.0	0.6
GA 3.0	0.6
GA 10.0	0.6

[1] 15 explants per treatment

Rooting. A rooting experiment was conducted to determine if simultaneous rooting and acclimatization is feasible for micropropagated shoots. Quick-dipping shoots for 5 min. in 500 mg/l K-IBA resulted in 10% rooting; the 1000 mg/l treatment resulted in 20% rooting.

DISCUSSION

Sterilization. Sterilization procedures must include a pre-sterilization treatment. This should include the use of systemic fungicides, such as Banrot and Subdue, as well as growing stock plants under greenhouse conditions. We observed that the use of a pre-sterilization treatment greatly increased the percentage of clean cultures.

Multiplication. Shoot multiplication was greatest with 7.5 mg/l BA, though there was not a significant difference between the 7.5 mg/l treatment and the 5 and 10 mg/l treatment. PBA (4) promoted bud break, but shoots were not as well developed or as numerous as those produced by the BA treatments. As the concentration of cytokinin increased, the number of shoots formed also increased. Toxicity symptoms such as thick, stunted shoots with curled leaves were observed at higher concentrations of BA. Only a very low number of shoots sufficiently elongated for easy handling during sectioning or rooting.

Elongation. There was very little difference among treatments in percentage of shoots greater than 5 mm. or average length of shoots greater than 5 mm. Before a commercial tissue culture propagation system can be implemented, greater elongation of the shoots must be achieved. This lack of elongation may be due to the absence of endogenous GA. Since there are no roots to produce GA (7), the mechanism to stimulate shoot development and elongation is reduced. By incorporating GA and BA into the basal medium, this problem may be overcome. Experiments are currently underway to determine if this is the case.

Rooting. From the rooting experiment, it appears that micropropagated *Sophora secundiflora* has the potential to root. Roots were observed in approximately 4 wks. No callus was observed in this treatment so higher concentrations of auxin may be used to increase rooting. It was also observed that the larger shoots rooted quite readily, while the shorter shoots did not root. This is probably due to the better contact of the longer shoots to the peat pellet. More rooting experiments are being conducted to determine the optimum treatment for rooting.

CONCLUSION

Using the *in vitro* micropropagation techniques as explained in this paper, we have a potential basis for clonal propagation of Texas mountain laurel. The ability to propagate *Sophora secundiflora* clonally will enable propagators to select for specific characteristics and mass produce certain genotypes. By having specific cultivars available, Texas mountain laurel will be more marketable as an ornamental landscape plant for the south.

LITERATURE CITED

1. Briggs, Bruce A. and Steven McCullouch. 1982. Preparation of plants for micropropagation. *Proc. Inter. Plant Soc.* 32: 297-301.
2. Briggs, Bruce A. and Steven McCullouch. 1983. Progress in micropropagation of woody plants in the United States and Western Canada. *Proc. Inter. Plant Prop. Soc.* 33: 239-241.
3. Chalupa, V. 1984. *In vitro* propagation of oak (*Quercus robur*) and linden (*Tilia cordata*). *Biologia Plantarum.* 26(5): 374-377.
4. Davies, F.T. and B.C. Moser. 1980. Stimulation of bud and shoot development of Reiger begonia leaf cuttings with cytokinins. *Jour Amer. Soc. Hort. Sci.* 105(1): 27-30.
5. Lloyd, G. and B. McCown. 1980. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture, *Proc. Inter. Plant Prop. Soc.* 30: 421-427.
6. McCown, B. and Ron Amos. 1979. Initial trials with commercial micropropagation of birch selections. *Proc. Inter. Plant Prop. Soc.* 29: 387-393.
7. Sebanek, J., F. Kopecky, and K. Slaby. 1978. Content of endogeneous gibberellins in pea roots in relation to their polarity. *Biochem. Physiol. Pflanzen.* 173: 448-450.
8. Sutter, Ellen and Philip Barker. 1983. Tissue culture propagation of selected mature clones of *Liquidambar styraciflua*. *Proc. Inter. Plant Prop. Soc.* 33: 113-117.
9. Welsh, K., K.C. Sink, and H. Davidson. 1979. Progress on *In vitro* propagation of red maple. *Proc. Inter. Plant Prop. Soc.* 29: 382-386.
10. Wood, B.W. 1982. *In vitro* proliferation of pecan shoots. *HortScience.* 17(6): 890-891.