SWEETGUM TISSUE CULTURE

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American sweetgum (*Liquidambar styraciflua*) is found naturally from as far north as Connecticut, southward into Florida and as far west as Texas. It can also be found in montane regions of Mexico and Central America. Due to the expansive and climatically diverse native range of this species, numerous ecotypes have been reported that exhibit greater frost tolerance, greater cold hardiness, differences in response to photoperiod, and differences in date of spring bud break and fall leaf retention (4, 8, 9). Great variation also exists in a number of ornamental characteristics, such as fall foliage color, fruitlessness, and form.

Sweetgum is commonly propagated by seed, but genetic variation and seed source variation make sexual propagation less than ideal in many instances. When asexual propagation must be used to propagate any of the numerous sweetgum cultivars, propagators usually have to employ grafting onto seedling rootstocks, since cutting propagation is rarely viable on a commercial level. Although grafting of sweetgum selections is usually very successful, grafted plants are not always the best landscape plants. Rootstocks often sucker freely, and cold-hardy cultivars, such as 'Moraine', grafted onto seedlings from southern seed sources, may survive a northern winter well, while the rootstocks freeze out from under the scions. In addition, grafted plants are still plagued by the variability imparted to them by the rootstock. Genotypes selected for non-invasive root systems, for example, cannot be propagated by grafting.

Micropropagation offers the propagator a viable and attractive alternative to grafting when one is asexually propagating sweetgum. Several unique aspects of *Liquidambar* should be kept in mind when one attempts to micropropagate this genus, and these will be outlined in the sections that follow. Most of my work has been with cold hardy cultivars, *L. styraciflua* 'Moraine', and *L. styraciflua* 'Variegata', and *L. styraciflua* 'Rotundiloba'. The difficulties I have experienced in working with these cultivars appear to be most pronounced in these, and other, northern types and less of a problem with clones of southern origin or with juvenile material.

Explant and Sterilization. Shoot tips harvested from actively growing shoots are a principal means of initiating shoot proliferating cultures of many woody species, including sweetgum (7). Shoot tips collected in the spring respond better than those collected in the summer and early fall (93% survival vs. 10% survival, respectively) (7).

Brand and Lineberger (1) have used lateral buds to initiate shoot proliferating cultures of mature-phase tissue. Lateral buds taken from the fifth or sixth node from the apex of an expanding shoot are in the proper physiological state to initiate *in vitro* growth and, in my experience, are superior to shoot tips. Buds taken with a shield of stem tissue initiate cultures readily, and rarely display the necrosis and need for rapid initial transfer typical of shoot tip explants. Within 2 to 3 weeks after explantation, the primary bud and two collateral buds begin to expand.

Initial sweetgum explants can be readily surface sterilized in a 10% Clorox solution for 15 min., followed by sterile distilled water rinses. Contamination rates for greenhouse-grown material collected in the spring can be expected to range from 5 to 10%. I have encountered occurrences of what appear to be endophytic bacterial contamination.

Shoot Proliferation. Woody Plant Medium (3) is the most suitable medium for sweetgum shoot proliferation. Murashige and Skoog medium (MS) (5) does not support acceptable sweetgum growth, often inducing necrosis and browning. A number of cytokinins have been tried with sweetgum, with benzyladenine (BA) at concentrations at or close to 1 mg l^{-1}, producing the greatest number of usable shoots. Shoot proliferating cultures of *Liquidambar* develop what I have termed "long shoot-short shoot" growth. What typically develops is a clump of a dozen or so short shoots, with 3 to 5 longer, well-expanded shoots. Short shoots are difficult to handle when they are treated as microcuttings.

Numerous treatments were tried on sweetgum that are known to enhance shoot elongation in other species, but none worked satisfactorily with *Liquidambar*. Gibberellic acid, various carbohydrate sources, low light levels, low cytokinin levels, and charcoal all failed to stimulate usefully significant shoot extension and concomitant leaf expansion. Addition of charcoal, removal of BA, or long subculture cycles stop shoot growth and stimulate rooting and the formation of a well-defined terminal bud.

Perhaps the best way to prevent the cessation of shoot growth and enhance shoot extension is to transfer cultures to fresh medium at 3 to 4 week intervals. Longer periods between subculture arrest the growth momentum of the shoots and send the culture into a

state which is somewhat analogous to "summer dormancy". A suggested transfer protocol to optimize formation of large microcuttings entails transfer of cultures to fresh medium after 3 weeks, followed by another transfer to fresh medium after 3 weeks and harvest of microcuttings after 5 weeks of growth on the last medium. The unharvested portions of the culture can then be put back into the cycle at the beginning.

The pH of shoot proliferation medium should be maintained at 5.2 or 5.3 and sucrose, at 2 or 3%, should be used as the carbohydrate source. A number of agars, including Sigma, Difco Bacto, and TC agar have worked well as solidifying agents for sweetgum cultures when used in the range of 0.6% to 0.8%. Gelrite or Phytagel used as solidifying agents result in poor quality cultures. Liquid culture of sweetgum results in severe vitrification and "lettuce-leaf" if shoots are immersed in the medium. Valuable cultures contaminated with bacteria can be successfully grown on polyester batting "barges" soaking in liquid medium. This type of culturing washes away damaging bacterial populations from the shoot clusters (without inducing vitrification) and enables production in cases where contaminated cultures would be killed or inhibited on agar solidified medium.

Environmental conditions which support good growth of *Liquidambar* cultures are: 23 to 25 °C; 14 to 16 h photoperiod; and 20 to 40 μE m^{-2} s^{-1} produced by cool white fluorescent lamps.

Culture Storage. Shoot proliferating cultures of sweetgum can be easily stored under refrigerated temperatures. I have stored shoot proliferating cultures of seedlings and named cultivars in the dark at 4 ± 2 °C continuously for 6 years without any loss of vitality when cultures are returned to typical culture conditions. Stored cultures continue to grow slowly under dark, refrigerated conditions, and develop etiolated, "spaghetti"-like growth. Cultures quickly green, and resume normal growth when retrieved from storage. Optimally, cultures should be transferred to fresh medium every 5 to 6 months during storage, but can go for 12 months or longer between transfers without serious losses in viability.

Microcutting Rooting and Plantlet Establishment. Sweetgum shoots produced *in vitro* can be easily rooted, either aseptically or under non-sterile conditions, and will go on to produce healthy plantlets. Even if they are short, robust shoots with thick stems and leaves along the entire length of the shoot make better microcuttings than long, spindly shoots with only a whorl of leaves at the apex.

Rooting microcuttings under non-sterile conditions can be an easy way to obtain sweetgum plantlets. With a pre-dip in 200 mg l^{-1} indolebutyric acid (IBA), rooting percentages of 70 to 90% for

mature material can be obtained (2). *In vitro* rooting is generally more consistent than non-sterile rooting, but is more resource-intensive. Half-strength salts are superior to full-strength salts (7), and I have rooted hundreds of sweetgum microcuttings using half-strength WP medium.

For juvenile shoots, the addition of IBA at 0.5 mg l^{-1} to the culture medium yields optimum rooting (7). For mature shoots, IBA in the range of 0.5 to 1.0 mg l^{-1} supports excellent root initiation, with the higher concentrations decreasing the time until rooting and increasing the number of roots per shoot. Providing microcuttings with auxin for a 3 to 4 weeks period, followed by transfer to hormone-free medium, enhances root initiation and subsequent root growth and elongation (1).

Acclimation of rooted sweetgum plantlets is usually accomplished by gradually reducing humidity and increasing light intensity. Sweetgum plantlets can be acclimated to the greenhouse by providing 7 days in shaded intermittent mist, followed by 7 days in a shaded greenhouse, or by substituting the week of intermittent mist with a gradual opening of the humidity chamber (1). During the rooting process, microcuttings form what is analogous to a summer dormant bud. Careful handling of acclimated plantlets is important, to insure growth of the apical bud and survival of the plants. It is best to acclimate plants when the daily photoperiod is increasing and relatively long; this means bringing rooted plantlets to the greenhouse in late winter to late spring. Plantlet survival in the spring and summer can be expected to be approximately 90% or better, but could be considerably lower than 50% in the winter. The use of HID lamps to extend the photoperiod will not overcome poor performance of plantlets in the winter, indicating that high light intensity, along with long photoperiods, are necessary to initiate growth in young plantlets. Ample fertilizer and water during the post-acclimation period also helps to induce a new flush of growth.

Once acclimated plants have resumed growth in the greenhouse, they can be expected to reach heights of 1 to 1.5 m and have stem calipers of 12 to 14 mm in 5 to 6 months, under accelerated growth conditions (2). Sommer and coworkers (6) determined that plantlets grown outdoors in a nursery bed for one year attain heights and stem diameters suitable for field planting. I have overwintered over 500, 6-month-old tissue-cultured plants in an unheated polyhouse and survival was nearly 100%. After one growing season in a container nursery area, these plants reach heights in excess of 2 m. Numerous plants have been planted in landscapes and in test plots. All plants appear to grow normally and at a rapid rate. We have not seen any indications of suckering (often seen on grafted plants) or poor root systems on any micropropagated plants.

Shoot Organogenesis from Leaf Tissue. When tissue of a particular sweetgum genotype is limited, shoot organogenesis from leaf pieces offers an means of rapidly increasing propagation stock. Shoot organogenesis requires relatively high levels of BA (2.5 mg l^{-1}), but is very reliable, both with greenhouse- and *in vitro*-produced leaves. The risk of producing "off-type" plants through adventitious shoot formation on leaves appears to be low. Shoot organogenesis on leaves has been thoroughly described (2).

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