

A Tissue Culture Solution to a Forestry Problem—The Propagation of a Tetraploid European Aspen

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A simple tissue culture method based on the production of multiple shoots from dormant buds is described. The application of the method to tetraploid European aspen (Populus tremula L.) illustrates that in vitro propagation can be an attractive alternative when conventional methods prove unsuccessful. Tree Planters' Notes 39(3):28-30; 1988.

Tissue culture has been proposed as a method for the large-scale clonal propagation of forest species. To date, *in vitro* propagation techniques have not been developed to the extent necessary for commercial forestry operations. Nevertheless, propagation of forestry species from tissue cultures may provide solutions to other forestry problems not directly related to mass propagation. The history of the propagation Ta-10, a tetraploid European aspen (*Populus tremula*) represents a case in point.

Hybridization of native, diploid quaking aspen (*P. tremuloides* Michx.) with tetraploid European aspen results in the formation of triploid aspen that are highly valued by the pulp and paper industry for their increased growth rate, specific gravity, and fiber length (2). Ta-10 originated in southern Sweden and was first crossed with native aspen at The Institute of Paper Chemistry (IPC) in 1958.

Since then, Ta-10 that has been grafted in the United States has been crossed with numerous *P. tremuloides* to the extent that up to 500,000 to 1,000,000 triploid hybrid seeds have been produced annually for the past 20 years. Although Ta-10 can be readily grafted onto diploid or triploid rootstock, attempts have been made at vegetative propagation. As a member of the section *Leuce*, Ta-10 is not easily propagated from hardwood cuttings but is amenable to propagation via root sprouts. Attempts to stimulate roots on Ta-10 by burial of grafts below the graft union were successful, but sprouting from those roots in the particular clone was minimal.

Therefore, tissue culture techniques became an attractive option to circumvent these difficulties in vegetatively propagating Ta-10. The following describes a general procedure that has been successfully used to propagate not only Ta-10 by tissue culture, but also other hardwood species as well.

Materials and Methods

Dormant lateral buds of Ta-10 were collected in January and February from the IPC arboretum near Greenville, WI. Buds were rinsed under cold water for 30 minutes and treated for 15 minutes with a 10% (v/v) solution of commercial bleach (Hilex). After three rinses with

sterile water, the bud scales and outer leaves were aseptically removed, and the apical meristems with several layers of intact leaf primordia were again treated with 1% bleach for 5 minutes. Following three rinses with sterile water, the explants were placed on woody plant medium (WPM) (3) containing 0.05 mg/liter naphthaleneacetic acid (NAA) and 1.0 mg/liter benzyladenine. The medium was adjusted to pH 5.8 prior to autoclaving and solidified with 0.8% agar (Bacto, Difco). The cultures were incubated at 22 °C and 3000 lux (cool-white fluorescent; 16/24-hour photoperiod).

Every 2 to 3 days, the explants were transferred to renewed medium by sliding them to a different portion of the petri dish. Every 2 weeks, the explants were subcultured to dishes of fresh medium. After 6 to 8 weeks "bud break" occurred, and shoots formed and multiplied. After 4 months, stable "shoot cultures" (fig. 1) could be maintained on the above medium without NAA. These cultures provided a continuous source of shoots suitable for rooting.

Root formation was accomplished *in vitro* as previously described (5). Briefly, one-third-strength medium (macro and microelements) containing 0.1 mg/liter indole butyric acid was used. Alternatively, shoots were transferred directly from



tissue culture to a mist bed for simultaneous rooting and hardening. Shoots or rooted plants were transferred to a soilless mixture containing equal parts sand peat, and perlite and watered to saturation with 1 liter benomyl (Benlate). Once established in soil, plants assumed growth rate and characteristics consistent with plants obtained from root sprouts (fig. 2).

Results and Discussion

Tetraploid European aspen could be readily propagated through tissue culture. Cultures were established that provided a year-round source of rootable shoots. Both bud break and root formation *in vitro* were nearly 100%. The procedure developed is not new but represents some of the best elements of several procedures for propagating aspen (1, 5) and sweetgum (4).

In the case of Ta-10, tissue culture provided a convenient means of vegetative propagation where several methods proved unsatisfactory. Situations similar to that encountered in Ta-10 can also arise in *Leuce* poplars in which clones have been maintained by grafting but the ortet has been lost. In most hardwoods the establishment of shoot cultures from lateral meristems is relatively simple and straightforward. For species that are difficult to root, the tissue

Figure 1—Shoot culture of tetraploid European aspen ($\times 3$).



Figure 2—Tissue culture-derived tetraploid European aspen 10 weeks after transfer to potting medium.

culture systems can be considered as providing material at the requisite physiological stage (i.e., rejuvenated) for root formation. Aside from grafting, tissue culture in difficult-to-root species may provide an attractive method of vegetative propagation.

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