PROPAGATION OF AMERICAN CHESTNUT IN VITRO

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ABSTRACT.--Axillary shoots developed on mature embryos of American chestnut seeds which were cultured on 6-benzylaminopurine (BAP-supplemented Murashige and Skoog (MS) medium. These shoots could be multiplied by subculture on MS medium with reduced BAP concentration. Rooting of some of these shoots occurred when they were placed on 3-indolebutyric acid (IBA)-supplemented medium followed either by culture on IBA - free medium, or by planting in vermiculite and placing them in a greenhouse mist propagation bed. Plantlets have been successfully transferred to soil in pots and grown outdoors. Axillary shoots have also been obtained from buds of 4-month-old greenhouse-grown seedlings.

Although it has received less attention in recent years, the selection and breeding of chestnut trees has been continued by several researchers and organizations (Given and Haynes 1978; Jaynes 1978; Keys et al. 1975; Thor 1978). The goal of these programs is to produce blight-resistant, timber-type chestnuts. In order to multiply selections or hybrids for outplanting, a reliable, inexpensive, and rapid method of asexual propagation is necessary. The techniques which have been used to propagate chestnut are either inadequate or are too costly (Keys 1978).

One possible technique would be the propagation of chestnut using in *vitro* shoot culture. Plantlet formation has been reported in apple (Jones et al. 1977), almond and almond-peach hybrids (Tabachnik and Kester 1977), European alder (Hosier et al. 1981), cherry (Ivanicka and Pretova 1980), and plum (Hammerschlag 1981) using the shoot culture technique. Vieitez and Vieitez (1980) reported plantlet formation of European chestnut. Keys and Cech (1981) reported the *in vitro* plantlet formation of American chestnut using shoots of embryonic origin.

This paper describes the methodology used in the culture of embryonic shoots and seedling buds of American chestnut. Results of various attempts to improve the rooting of these shoots and establishment of the resulting plantlets will be discussed.

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Culture Initiation

Shoot cultures were initiated using excised embryos of stratified American chestnut seeds. The seeds were stratified in moist sand in plastic bags for 60 days at 1 C. Surface sterilization was accomplished by dipping the seeds in 95 percent ethanol for 1 minute, followed by flaming and cooling in sterile distilled water. The seed coat was then cut and peeled away. The embryo was excised and placed radicle-end-down on 30 ml of nutrient agar medium in a 25 x 150 mm culture tube which was then sealed with aluminum foil.

Buds of greenhouse-grown American chestnut seedlings were also used to initiate shoot cultures. Stem sections were cut and soaked overnight in distilled water, with 0.1 percent Alconox as a wetting agent. The stem sections were surface sterilized by soaking in 5 percent sodium hypochlorite (100 percent commercial bleach solution) for 10 minutes followed by three rinses in sterile distilled water. The stems were cut into 1 cm - long sections, each having one bud. Each explant was placed vertically on 50 ml of nutrient agar medium in a 125 ml Erlenmeyer flask which was then sealed with aluminum foil. Both types of cultures were grown in growth chambers at 27 + 2 C under a 16-hr photoperiod of 200 fc of fluorescent and incandescent light. After 4 to 6 weeks, a whorl of axillary shoots developed at the base of the original explants.

The initiation medium consisted of Murashige and Skoog (MS) (1962) macro and micro nutrients supplemented with (per liter) 0.5 mg niacin, 0.5 mg pyridoxine•HC1, 0.1 mg thiamine•HC1, 100 mg myo-inositol, 30 g sucrose, and 1.0 mg 6-benzylaminopurine (BAP). The pH of the nutrient medium was adjusted to 5.5 to 5.6 with IN NaOH prior to filtering and autoclaving. The medium was solidified with 6 g/1 Phytagar (Gibco).

Shoot Multiplication

Individual shoots which were at least 1.5 cm long, or clumps of shoots, were excised from the original explant and subcultured in order to further multiply the number of shoots. The subcultures were placed on 50 ml of nutrient medium in 125 ml Erlenmeyer flasks and were grown under the same conditions as the original explants. The shoot multiplication medium was the same as the culture initiation medium except that the nitrates were reduced by half, and only 0.1 mg/1 BAP was added.

New shoots formed in 4 to 6 weeks. Average shoot production was approximately 10 per culture. The ability to produce axillary shoots did not decrease, even after eight subcultures. In this way, as many as 800 to 1000 shoots per year could have been produced from each original explant.

Root Initiation

Shoots which were at least 1.5 cm long were subcultured to a root induction medium. This medium consisted of MS salts with the nitrates reduced by one-half, and supplemented with 1.0 or 3.0 mg/1 of 3-indolebutyric acid (IBA). The shoots were maintained for 1 or 2 weeks on this medium in the previously described environment. After this period, the shoots were subcultured to the

same medium without hormones to allow roots to develop. Rooting occurred in 4 to 6 weeks (Figure 1). At present, the methods tested for root initiation have not produced adequate results. Rooting has occurred on only 4 percent of the shoots which were subcultured to the root induction medium. Further tests showed that concentrations of IBA above 3.0 mg/1 stimulated excessive callusing at the base of the shoots, which inhibited root development. Tests using 3-indole acetic acid (IAA) or naphthaleneacetic acid (NAA) instead of IBA failed to induce rooting.

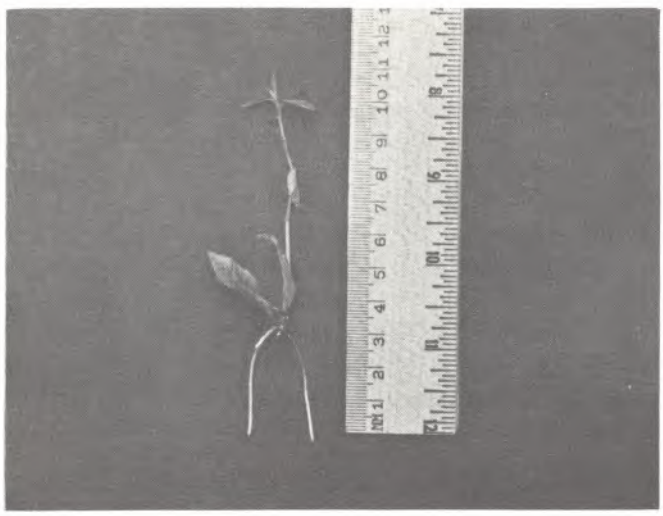

Figure 1. American chestnut plantlet produced *in vitro*.

Another technique showed slightly better preliminary results, with 17 percent (3 of 18) of the shoots rooting. One of the shoots that rooted using this technique was of seedling origin. The shoots were dipped for 1 second in 5000 mg/1 of IBA in 95 percent ethanol, followed by culture on hormone-free, reduced-nitrate medium. Basal callusing was severe on many of the shoots, indicating that the concentration of IBA might have been too high. The same technique using IAA failed to induced rooting.

Establishing Plantlets in Soil

The transfer of the rooted shoots from the sterile culture condition to soil outdoors had to be done gradually. The plantlets were removed from the medium and the roots were washed to remove any remaining agar. They were then planted in vermiculite which had been soaked with half-strength MS salts. The plantlets were placed on a greenhouse propagation bed under intermittent mist until the roots were well developed (at least 3 weeks). After this time, they were transferred to soil in pots and grown outdoors under a shade house. The root system had to be well-developed, or the plantlets died during this stage. One plantlet was successfully transferred to soil using this method. After 4 weeks in soil, it was vigorous and putting on new growth.

Conclusions

American chestnut shoots from juvenile tissue can be rapidly and easily multiplied *in vitro.* As has been the problem with chestnut in the past, a reliable method of rooting these shoots must be developed. However, since the shoots are succulent and physiologically juvenile, the possibility of developing such a rooting technique is good. Other difficulties may exist in transferring the plantlets to soil for growth under normal environmental conditions. And the technique has yet to be tested using buds from mature trees. Once these problems are overcome, shoot culture may be a good tool for the multiplication of blight-resistant or superior American chestnut or hybrid clones.

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