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In Vitro Proliferation of Pecan Shoots[1]

B. W. Wood[2]
U.S. Department of Agriculture, ARS, Southeastern Fruit and Tree Nut Research Laboratory, P.O. Box 87, Byron, GA 31008

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Abstract. **Pecan [*Carya illinoensis* (Wang.) Koch] axillary buds of nodal explants from 4- to 12-week-old 'Stuart' seedlings were first induced to proliferate shoots and then to elongate on a defined medium. An evaluation of 6-benzylamino purine (BA), isopentenyladenine (2iP), indolebutyric acid (IBA), and indoleactic acid (IAA) for shoot proliferation found a combination of 4 mg/liter BA and 1 mg/liter IBA to be most effective. The synthetic hormones were much more effective at inducing a growth or development response than were their natural counterparts. Gibberellic acid (GA) at 3 mg/liter plus 0.1 mg/liter BA enhanced shoot elongation.**

Pecan cultivars are propagated by either grafting or budding onto seedling rootstocks. Successful rooting of cuttings and subsequent field survival has been limited (1, 5, 6). Pecan rootstocks are propagated by seed and consequently are undesirably heterozygous. A culture method that could produce large amounts of genetically uniform plants, either own-rooted cultivars or clonal rootstocks, would be important for the commercial production of plants and for rootstock research.

Tissue culture techniques have been developed for several tree crops, but previous efforts with pecan have shown that it is difficult to propagate by *in vitro* methods. To date, results have been limited to the formation of callus with only limited shoot and root formation (3, 7); no consistent shoot proliferation, elongation, or root development occurred. The purpose of this research was to determine the conditions necessary for *in vitro* proliferation of axillary shoots.

Nodal sections of 4-week-old greenhouse-grown seedlings from open-pollinated 'Stuart' seed were excised so that each section contained 1 visible bud per explant. Sections (1 to 2 cm) were collected in distilled water with 0.5% Tween-20, surface-sterilized with 1.0% sodium hypochlorite (20% commercial bleach) for 20 min, and rinsed 5 times in sterile distilled water. Explants were then placed on prepared agar medium in 150 × 25-mm culture tubes.

The basic culture medium consisted of the nutrient formulation (Woody Plant Medium) developed by McCown and Lloyd (4). Medium composition was (in mg/liter), 400 NH_4NO_3, 556 $Ca(NO_3)_2 \cdot 4H_2O$, 990 K_2SO_4, 96 $CaCl_2 \cdot 2H_2O$, 170 KH_2PO_4, 6.2 H_3BO_3, 0.25 $Na_2MoO_4 \cdot 2H_2O$, 370 $MgSO_4 \cdot 7H_2O$, 22.3 $MnSO_4 \cdot H_2O$, 8.6 $ZnSO_4 \cdot 7H_2O$, 0.25 $CuSO_4 \cdot 5H_2O$, 27.8 $FeSO_4 \cdot 7H_2O$, 37.3 $Na_2 \cdot EDTA$, 1.0 thiamine · HCl, 0.5 nicotinic acid, 0.5 pyridoxine · HCl, 2.0 glycine, 100 myo-inositol, 20,000 sucrose, and 6,000 Phytagar. The pH was adjusted to 5.2 prior to the addition of agar.

Pecan tissue, especially older tissue, often results in contaminated cultures after rigorous surface-sterilization procedures because endophytic organisms are contained within the tissue (2). Growth of these internal contaminants was partially controlled by adding 200 mg/liter streptomycin to the medium before autoclaving (121°C for 15 min), and 40 mg/liter filter-sterilized Pimarcin added to the sterilized media before gelling.

For shoot proliferation, filter-sterilized cytokinins were added to the media before gelling. BA and 2iP were compared at 0, 0.5, 1, 2, 4, 8, 16, and 32 mg/liter for suitability for inducing shoot proliferation. The study was designed as a randomized complete block with 8 concentrations of each cytokinin replicated 8 times. Each treatment consisted of 5 explants as the experimental unit.

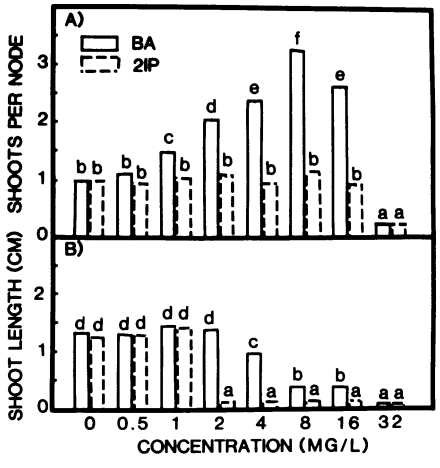

Fig. 1. The influence of BA and 2iP on A) shoot proliferation and B) shoot elongation from nodal explants of pecan seedlings. Mean separation by Duncan's multiple range test, 5% level.

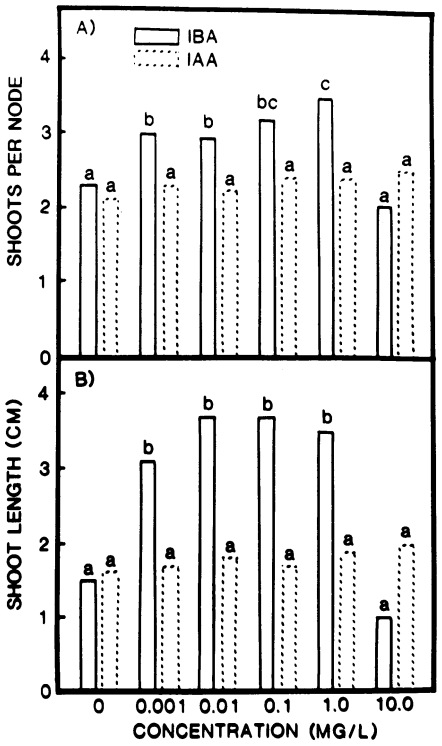

Fig. 2. The influence of IBA and IAA in a basic medium containing 4 mg/liter BA on A) shoot proliferation and B) shoot elongation from nodal explants of pecan seedlings. Mean separation by Duncan's multiple range test, 5% level.

The induction of shoot proliferation was further investigated by adding either IBA or IAA to the basic medium with 4 mg/liter BA. IBA and IAA were tested to 0, 0.001, 0.01, 0.1, 1, and 10 mg/liter in 4 replicates in a randomized complete block design with 5 explants as the experimental unit.

Subsequent shoot elongation was accomplished by using the above basic medium with 0.1 mg/liter BA and varying the levels of filter-sterilized gibberellic acid (GA). The experiment was designed as a randomized complete block with 6 replicates of GA treatments at 0, 0.5, 1, 2, 4, 8, and 16 mg/liter. Experimental units consisted of 5 explants.

Explants were grown in a growth room

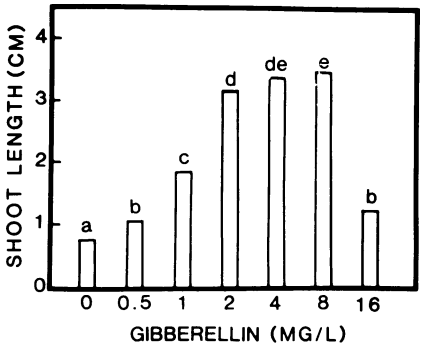

Fig. 3. The influence of GA on elongation of shoots from explants of pecan seedlings. Mean separation by Duncan's multiple range test, 5% level.

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[2]Research Horticulturist.

Fig. 4. Pecan shoots produced from axillary buds of seedlings, using 4 mg/liter BA plus 1 mg/liter IBA for shoot proliferation and 8 mg/liter GA plus 0.1 mg/liter BA to induce shoot elongation.

with 50–75 $\mu Em^{-2}s^{-1}$ of photosynthetically active radiation for 16 hr/day from Gro-Lux fluorescent lights. Temperatures ranged from 22 to 25°C. Explants were incubated from 3 to 6 weeks and evaluated. Periods of incubation on the same medium greater than 4 weeks resulted in a definite decline in explant vigor.

Shoots developing from each node increased as BA levels increased to 8 mg/liter with a maximum of 3 shoots produced by the 8 mg/liter BA treatment. Fewer shoots were produced at higher BA concentrations and the explants died on 32 mg/liter. The 2iP treatments failed to stimulate shoot proliferation and 32 mg/liter was toxic (Fig. 1-A).

Shoot length was unaffected by cytokinin levels up to 2 mg/liter for BA and 1 mg/liter for 2iP, but was reduced at higher concentrations (Fig. 1-B). Concentrations of BA above 1 mg/liter produced longer shoot growth than did 2iP, but growth was less than the controls. As BA increments increased, shoots developed a progressively redder color. Optimum shoot proliferation was at 4 mg/liter BA. BA at 8 mg/liter produced the greatest number of shoots per node, but they were very short and appeared to be injured, however, they would elongate and grow when transferred to the shoot elongation media.

The addition of IBA to the medium, in addition to 4 mg/liter BA, resulted in an increase in both the number of shoots per node and shoot length with concentration up to 1 mg/liter of IBA (Fig. 2). IAA did not affect either shoot number or length.

The synthetic cytokinin (BA) and auxin (IBA) were far more biologically active than were either native compound (2iP and IAA) which either failed to induce a response or the response induced an unfavorable response.

When explants were cultured through the proliferation phase with 4 mg/liter BA for 6 weeks, then transferred and grown 4 weeks in a medium containing 0.1 mg/liter BA with varied GA concentrations, shoot growth increased threefold with 2–8 mg/liter GA (Fig. 3). GA at 2 mg/liter was almost as effective at inducing shoot growth as was 8 mg/liter. GA was toxic at 16 mg/liter and resulted in reduced shoot elongation and leaf browning; levels from 0.5 to 8 mg/liter had no visual effect on morphology or color.

Pecan shoots can be produced from nodal explants of seedlings and can be induced to elongate in preparation for transfer to a rooting medium (Fig. 4). Since shoots appear to originate only from existing nodal buds rather than via adventitious bud formation, the number of shoots that can be produced may be limited. Attempts either to subculture shoots or to induce rooting have been unsuccessful.

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In Vitro Propagation of *Rhododendron catawbiense* from Flower Buds[1]

Martin M. Meyer, Jr.[2]

Department of Horticulture, University of Illinois, Urbana, IL 61801

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Abstract. **Flower pedicels and ovary bases of 4 cultivars of *Rhododendron catawbiense* Michx. were cultured *in vitro* and proliferated granular masses of tissue on Anderson's medium containing indoleacetic acid (IAA) 1.0 or 4.0 mg/liter and 6(γ,γ-dimethylallylamino)-purine (2iP) 5.0 or 15.0 mg/liter. These masses formed numerous shoots when cultured on Anderson's medium with lower levels of growth regulators. The shoots were rooted and grown as plants which appeared normal in vegetative characteristics.**

In vitro propagation of *Rhododendron* has been reported by Anderson (1, 2) and Kyte and Briggs (4) using a low K medium developed by Anderson (3). The shoot tips or small cuttings (2–3 cm) used for explants are very difficult to disinfest because of hairy, sticky surfaces and large size. The *in vitro* propagators of rhododendron obtain only 4–5% success after vigorous disinfestation. Stock plants often have to be raised in a greenhouse to obtain even this yield.

Shoot tip culture was tried on several *R. catawbiense* cultivars, but the tips from outdoor plants proved impossible to sterilize. Since the flowers of various herbaceous perennial plants have proved to be successful sources of explants for *in vitro* propagation (5) this technique was tried with *Rhododendron*. The flowers of *R. catawbiense* proved easy to obtain in a sterile condition because the flowers are borne in a sheath arrangement in a large bud and are covered with outer bud scales which are initiated before the flowers and form an outer covering to protect the flowers.

The following procedures were used for successful *in vitro* propagation of *R. catawbiense album*, 'Nova Zembla', 'Roseum Elegans', and 'Sefton', using flowers as explants. The flower buds were removed from the outdoor stock plants several times during the dormant period from October to April. The complete buds containing 15–20 florets were brought into the laboratory, washed with detergent, and the outer resinous bud scales were removed until the white, papery coverings of the florets were exposed. These buds were then immersed in a solution of 0.5% NaOCl (10% Clorox) and 0.1% po-

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[2]Associate Professor of Nursery Management.