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Somatic Embryogenesis in Tissue Cultures of Pecan

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Abstract. **Tissue cultures of pecan [*Carya illinoensis* (Wangenh.) C. Koch] were initiated from embryo explants collected weekly from 12 weeks post-pollination until fruit maturity. Three cultures derived from immature embryos collected 14 weeks post-pollination produced primary somatic embryos within 1 month following transfer from modified woody plant medium (WPM) with 2.0 mg·liter^{-1} 2,4-D and 0.25 mg·liter^{-1} BA in the light to hormone-free medium in the dark. Scanning electron microscopy documented the development of secondary embryos, which followed a globular, heart-shaped, and torpedo-stage developmental sequence. Chemical names used: (2,4-dichlorophenoxy) acetic acid (2,4-D), *N*-(phenylmethyl)-1*H*-purin-6-amine (BA), and 1*H*-indole-3-butanoic acid (IBA).**

Pecan is a tree species with important commercial value as a nut crop. Pecan is difficult to root, and problems of low and variable establishment exist (11); thus, cultivars are budded or grafted on open-pollinated seedling rootstocks. The extensive variability in yield and growth of pecan cultivars on different rootstocks has been realized for some time (10). Clonal propagation related to rootstock selection would have an important effect on crop improvement, as has been achieved with many other fruit crops (3). In addition, clonal material would be of value in nutritional and physiological research where genetic consistency is essential (8).

Previous tissue culture work with pecan has concentrated on micropropagation of cuttings obtained from seedlings (2, 4, 14) or buds of adult trees (9). Neither Wood (15) nor Phillips and Ramirez (9) were successful in obtaining rooting. Hansen and Lazarte (2) produced plantlets; however, shoot multiplication rates were low (1.5 shoots per explant in their best treatment). Knox and Smith (4) were unable to establish plantlets in soil derived from nodal stem explants.

Litz (6) has shown that many species of tropical fruit trees can produce somatic embryos in tissue culture. Somatic embryogenesis also has been reported in several temperate fruit species, including apple, grape, sweet cherry, and filbert (1, 12). Recently, Tulecke and McGranahan (13) reported somatic embryogenesis in members of the pecan family, Juglandaceae, i.e. *Juglans regia*, *J. hindsii*, and *Pterocarya* sp., using immature zygotic embryo explants. A distinct advantage of embryogenic culture systems is the higher multiplication rates than are obtained through organogenesis (1).

Developing fruit were collected from pecan trees of the cultivar Stuart, grown on the Univ. of Georgia Horticulture Farm, Watkinsville. Collections began 1 Aug. 1985 (about 12 weeks following pollination) and continued at weekly intervals for 11 weeks until maturity (shuck-split) during the 2nd week of October. Fruit were surface-sterilized using a 70% ethanol dip; 10% Roccal dip; 5-min immersion in undiluted Clorox (5.25% sodium hypochlorite); followed by a sterile water rinse, 0.01 N HCl rinse, and three additional sterile water rinses. Embryos were removed aseptically, and those <1 cm in length were left intact; after week 15, the embryos were cut into pieces ranging from about 0.3 to 2 cm in width and length. During early collection periods, the embryos were too immature to detect macroscopically; the micropylar end of the ovule, which contained the embryo, was removed and placed in culture. The explants were placed on one of two modified conditioning WPM (7), P1 or P2 (Table 1), with 30 g·liter^{-1} sucrose and 0.8% agar at pH 5.7 in 100 × 15 mm plastic petri dishes, sealed with Parafilm. Explants from 10 fruit were placed on each medium for each collection date. Cultures on P1 and P2 media were maintained under cool-white fluorescent bulbs at 70 μmol·s^{-1}·m^{-2}, on a 15-hr photoperiod. After 6–8 weeks, the cultures were transferred from conditioning to induction media (Table 1), which were the same as conditioning media but lacking hormones. Some explants from each culture also were maintained on conditioning media where possible. Cultures conditioned on P1 medium were transferred to P3 induction medium, while those on P2 medium were transferred to P4 induction medium. All cultures on P3 and P4 media were kept in the dark and subcultured to fresh media monthly. For morphological evaluations using scanning electron microscopy (SEM), tissue samples were fixed as previously described (14) and examined in a Phillips 505 SEM.

Explants cultured on P1 medium did not form a callus. About 30% of these cultures formed 10 to 20 solid, white nodules, ≈1 mm in diameter, on the adaxial surfaces of the cotyledons (Fig. 1A). When transferred

Table 1. Conditioning and culture media (milligrams per liter) used for somatic embryogenesis in pecan.[z]

Medium	Glutamine	Casein hydrolysate	2,4-D	Kinetin	BA	IBA
P1 conditioning	250	0	0	2.0	1.0	0.01
P2 conditioning	0	1000	2.0	0	0.25	0
P3 induction	250	0	0	0	0	0
P4 induction	0	1000	0	0	0	0

[z]Basal woody plant medium (WPM) of Lloyd and McCown (7)

Table 2. Embryo explant responses following 8 weeks on P4 medium.

Weeks post-pollination	Culture response (%)[z]			
	Green callus	White callus	Adventitious roots	Somatic embryos
12	20	10	0	0
13	30	0	0	0
14	20	10	0	30
15	30	10	0	0
16	30	0	0	0
17	60	10	40	0
18	70	30	60	0
19	90	80	30	0
20	70	50	30	0
21	100	10	20	0

[z]Percentages may total more than 100 due to multiple responses of cultures.

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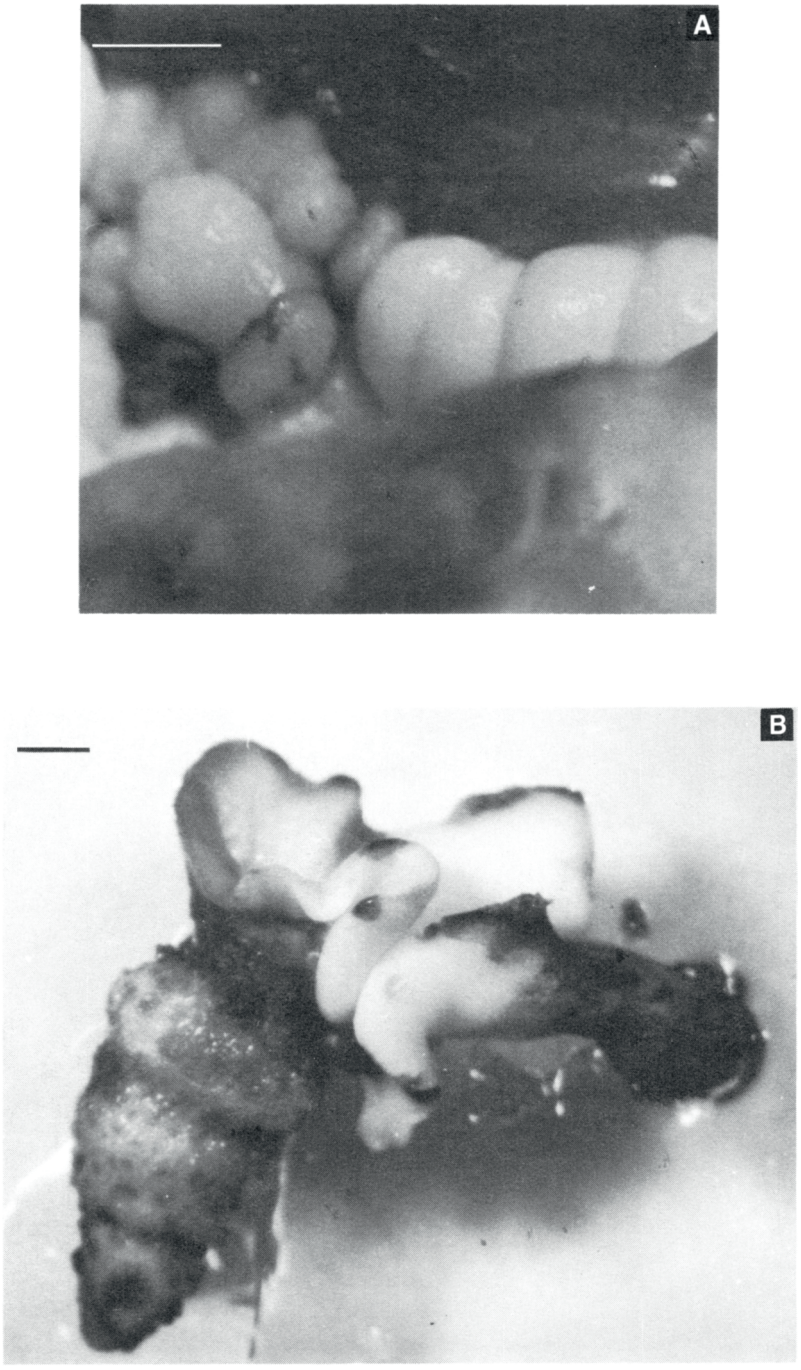

Fig. 1. Nodular structures and somatic embryos derived from cultures of immature pecan zygotic embryos. (**A**) Nodular structures on adaxial surface of zygotic embryo cotyledon. (**B**) Primary somatic embryo with thick roots and fused cotyledons. Bar = 1 mm.

to P3 medium, these nodules did not develop further and often produced a slow-growing white callus. Adventitious roots also emerged from these cotyledon pieces. Some embryos from the early August collection on P1 medium partially germinated and produced plants with stunted apices and cotyledons. These cotyledons also formed nodules on their adaxial surfaces.

Explants cultured on P2 medium formed a rapidly growing, dark-green callus that continued growth when placed on P4. After 4 to 8 weeks on P4, four general responses were observed: 1) continued growth of the dark green callus; 2) differentiation of a callus composed of white, translucent, spherical cells; 3) differentiation of adventitious roots; and 4) differentiation of somatic embryos (Table 2).

The cultures differentiating somatic embryos originated from intact zygotic embryos, which were placed on P2 14 weeks after pollination, transferred to fresh P2 after 1 month, and transferred to P4 3 weeks later. Embryoids were first separated from the callus and placed on fresh P4 in separate petri dishes in the dark. A few of the embryos produced roots and their fused cotyledons expanded partially (Fig. 1B). In other primary somatic embryos, only the cotyledons expanded. Root elongation was absent and secondary somatic embryos formed in the region of the radicle. SEM showed numerous secondary somatic embryos (Fig. 2A), which clearly followed the globular and heart-shaped sequence of zygotic embryo development (Fig. 2 B and C). Among those secondary embryos continuing development past the torpedo stage, two general forms appeared. In one form, the cotyledons remained fused into a tube, similar to the primary somatic embryos. In the other, the cotyledons were separate and continued to expand with visible lobing (Fig. 2D).

After 4 months on conditioning P2 medium, some cultures of zygotic embryos collected in early August produced a pale green nodular callus. Although this callus was similar in appearance to proembryogenic masses described in embryogenic cultures of other species (5), no somatic embryos developed. None of the pecan culture lines differentiating somatic embryos produced nodular callus. The somatic embryos either originated from non-nodular callus or adventitiously from uncallused embryo tissue.

We thus have obtained somatic embryogenesis in pecan, although at a low frequency. Our preliminary results indicate that there may be an optimal period of zygotic embryo development for the induction of somatic embryos, as was found by Tulecke and McGranahan (13) for *J. regia*. In pecan, all somatic embryos and nodular (potentially embryogenic) callus originated from zygotic embryos collected 12–14 weeks post-pollination. Additional replications over several seasons are needed to prove the validity of the preliminary observations described.

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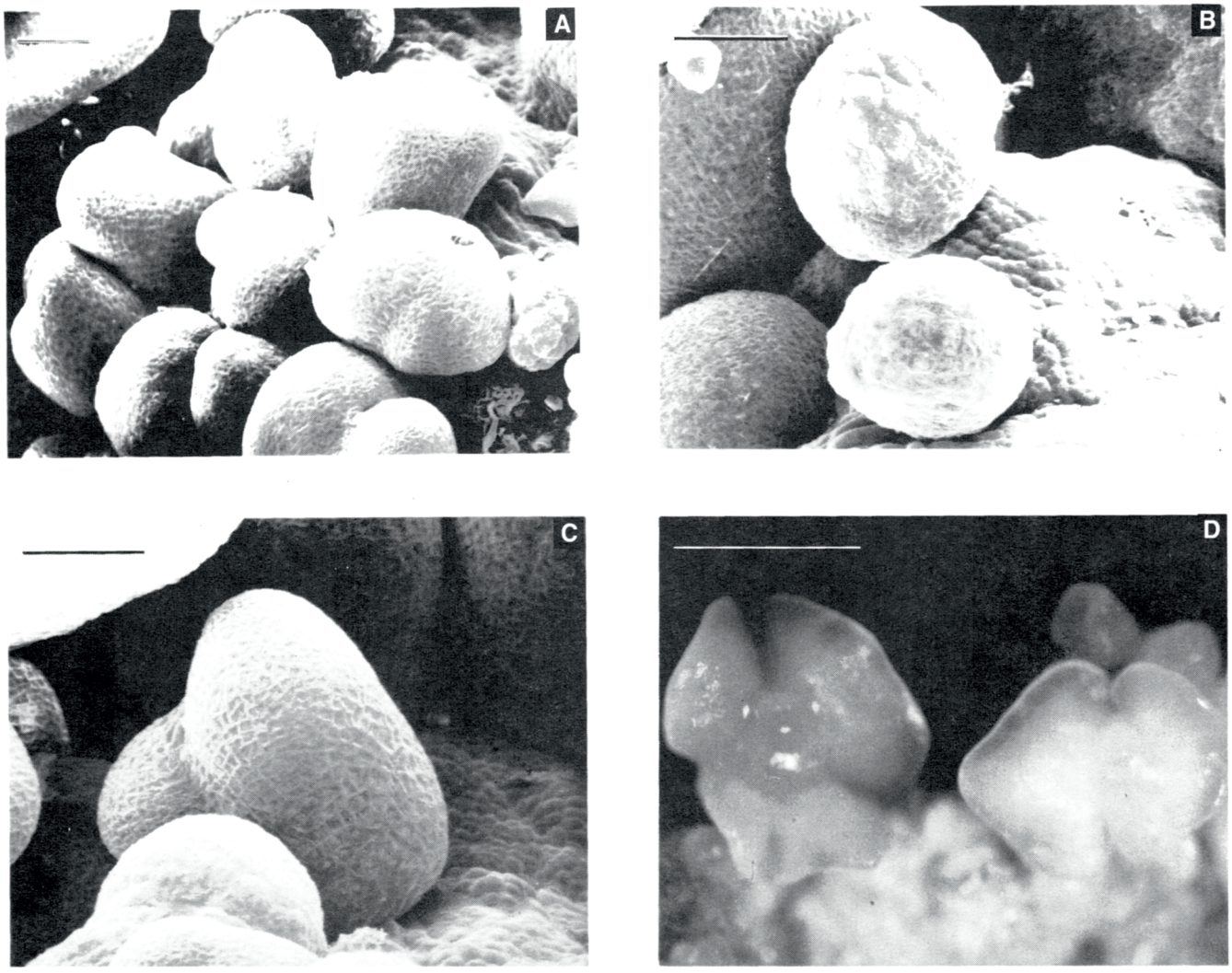

Fig. 2 Somatic embryogenic stages. (**A**) Globular, heart-shaped, and intermediate secondary somatic embryos. (**B**) Globular secondary somatic embryos. (**C**) Heart-shaped secondary somatic embryo. (**D**) Expanding secondary somatic embryos with lobed cotyledons. Bar = 100 μm.

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