Somatic Embryogenesis from Pecan Zygotic Embryo Explants

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In vitro somatic embryogenesis could be a highly efficient means for vegetatively propagating the pecan [*Carya illinoensis* (Wangenh.) K. Koch] (Merkle et al., 1987). Merkle et al (1987) induced somatic embryos from cultured immature zygotic embryo explants of 'Stuart' pecan. Wetzstein et al. (1988) obtained 40% induction of somatic embryogenesis with some explant stages of 'Stuart' and 'Desirable' pecans. In both cases, however, these somatic embryos failed to develop into complete plantlets. We report here the induction of somatic embryogenesis in two cultivars, with a low frequency of development into complete plantlets.

Immature fruit, at 16 weeks postpollination, were collected from open-pollinated trees of 'Western Schley' and 'Wichita' grown in an orchard southwest of Las Cruces, N.M. Embryonic axes attached to small basal portions of the developing cotyledons of the zygotic embryos were excised from the ovules under a dissection microscope. Excised tissues for explanting were surface-sterilized by submersing briefly in 95% ethanol and for 7 min in 2.6% sodium hypochlorite (50% commercial bleach), followed by two rinses in sterile deionized water. Four to five explants were cultured in 100 × 15-mm petri dishes, with 12 replications (dishes) per treatment. We used BDS basal nutrient medium (Dunstan and Short, 1977) supplemented with 0.51 mM ascorbic acid and the following growth regulator combinations: 0.05 μM IBA + 4.4 μM BA + 9.3 μM kinetin (Tulecke and McGranahan, 1985);

0.05 μM 2,4-D + 14.8 μM adenine (Phillips and Collins, 1980); 0.04 μM picloram (Dow Agricultural Products, Midland, Mich.) + 4.4 μM BA; or 0.41 μM picloram + 2.2 μM BA. Petri dishes were sorted randomly during incubation at 25 ± 0.5°C either in darkness or with a 16-hr photoperiod (15 μmol·s⁻¹·m⁻²). Cultures were transferred onto fresh media at 30-day intervals.

Zygotic embryo axis explants of both cultivars gave rise to globular somatic embryos directly and to embryogenic callus at similar frequencies. The globular somatic embryos failed to develop further; however, the embryogenic callus proliferated rapidly. The frequency of somatic embryogenesis was similar for both cultivars and light conditions. Growth regulators had a significant effect on the induction of embryogenic callus (Table 1). The medium containing 2,4-D was the most effective for induction of somatic embryogenesis. The induction frequencies observed were comparable to those observed by Merkle et al. (1987), but lower than those observed by Wetzstein et al. (1988). The embryogenic callus continued to proliferate during subsequent culture passages. Somatic embryos differentiating from the embryogenic callus exhibited limited development and maturation, similar to previous results (Merkle et al., 1987; Wetzstein et al., 1988).

During the first four passages, shoot development from somatic embryos was observed only on medium containing 0.04 μM picloram + 4.4 μM BA. Consequently, all

cultures were placed on medium of this composition. Over the next six passages, 7% of the cultures developed shoots from somatic embryos. Developing shoots were isolated and recultured on medium of the same composition. Shoot multiplication was thus maintained for several additional months by enhanced axillary branching, with propagation rates of four ('Wichita') to seven ('Western Schley') shoots per month obtained from each somatic embryo-derived shoot. Incompletely developed somatic embryos, but not the shoots, showed spontaneous root formation from 9% of the cultures on this same medium. Subsequently, the individual shoots isolated from the shoot multiplication cultures described were rooted with 49% frequency upon culture for 4 weeks on medium containing 14.8 μM IBA, producing complete plantlets.

This experiment demonstrated the induction of somatic embryogenesis in two additional cultivars of pecan, thus extending the genotype base for further studies and application. In contrast to previous reports, these results also indicated the potential to successfully obtain complete plants from pecan somatic embryos.

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Table 1. Frequency of embryogenic callus induction after 30 days of culture^a of zygotic embryo axis explants from 'Wichita' and 'Western Schley' pecans.

Growth regulator treatments used with BDS basal nutrient medium + 0.51 mM ascorbic acid	Percentage of explants giving rise to embryogenic callus ^c
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^aMean separation at *P* = 0.05 by Fisher's protected least significant difference test (Steel and Torrie, 1980).