BENEFITS

- More plants per acre.
- Can harvest on any day of the year.
- A container-grown plant that should not be root bound.
- Plants have the advantage that they are anchored in the ground and do not blow over in the wind.
- Overwintering uses the natural ground heat without the need to heal-in or move to a structure.
- Moisture and nutrients can be monitored better than in a field situation.

Adventitious Bud and Shoot Formation in Pawpaw [*Asimina triloba* (L.) Dunal] Using Juvenile Seedling Tissue

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INTRODUCTION

Current clonal propagation methods for the North American pawpaw [*Asimina triloba* L. Dunal] are limited to budding and grafting techniques (Layne, 1996). No work has been published detailing a micropropagation system for the pawpaw, but Callaway (1992) indicated limited success in regenerating shoots using leaf tissue. Successful micropropagation systems have been developed for related *Annona* species (George and Nissen, 1987). The objective of this research was to observe the effect of ontological age on adventitious bud and shoot development of pawpaw nodal explants in culture. Explants from a juvenile source (seedlings) and mature sources (forced stems and shoots produced on root pieces) were used to study the effect of ontogenetic age during the establishment phase.

MATERIALS AND METHODS

Seedling Explants. Stratified seeds were planted in vermiculite and germinated in a 25C growth chamber. Approximately 12 weeks after planting, seedlings had 6 to 10 nodes.

Mature Wood Explants. Dormant stems were collected from 26 genetically different mature, flowering trees and surface sterilized. Stems were forced in beakers in a 25C growth chamber. After 6 weeks, shoots had expanded to ≥ 10 cm.

Explants from Shoots Produced on Root Pieces. Root pieces (≥5 mm diameter, 10 to 12 cm length) were obtained from mature trees in a native stand. Shoots were forced on root pieces kept in a 22C greenhouse and after 20 weeks shoots had expanded to ≥ 10 cm.

Explant Preparation. Single node explants were excised from each source, washed and surface disinfested (10% Clorox for 10 min). After rinsing, single explants (1 to 3 cm in length) were inserted vertically and cultured on 20 ml of M.S. medium supplemented with 10 μM BA and 0.1 μM TDZ in 25 × 150-mm test tubes. Medium pH had been adjusted to 5.8 ± 0.1 prior to autoclaving and solidified with 0.6% Bacto-agar (Difco Laboratories, Detriot, MI). Culture tubes were maintained under 16-h photoperiod of 20 μmol sec^{-1} m^{-2} of light provided by cool white fluorescent bulbs. Culture room temperature was constant 25C.

Explant transfer and data evaluation occurred at 2-week intervals and at each interval, the percentage of explants with elongating axillary shoots was recorded. Axillary shoots were determined as the number of shoots (> 2 mm in length) arising from a leaf axil. Formation of adventitious buds (< 0.5 cm) and shoots (≥ 0.5 cm) was also recorded at each transfer interval. Adventitious buds and shoots were determined as buds and shoots arising at any location on the explant other than in a leaf axil.

RESULTS AND DISCUSSION

The effect of ontogenetic age on explant performance was seen with the inability of explants from 26 mature sources to respond in culture (Table 1). From 551 mature explants, 72% were successfully disinfested, but only 4% survived the culture environment. No explants from mature sources produced axillary shoots or adventitious buds. The small percentage of explants from mature sources that survived in the culture environment showed some tissue proliferation after approximately 7 months in culture.

Table 1. Percentage of North American pawpaw (*Asimina triloba*) explants showing axillary bud elongation of seedling, shoots produced from root cuttings, and mature source nodal explants after 4, 6, and 8 weeks on an MS medium supplemented with 10 μM BA and 0.1 μM TDZ.

	Weeks in culture		
Explant Source	4	6	8
Seedling (n=25)	60%	72%	100%
Shoots from root cuttings (n=42)	0%	0%	42%
Mature (n=551)	0%	0%	0%

Seedling explants, ontologically juvenile tissue, responded rapidly in vitro. After 4 weeks in culture, seedling explants had expanded axillary buds and after 6 weeks, the expanded shoots were suitable for subculture. At 8 weeks, axillary buds elongated on all seedling nodal explants and multiple adventitious buds and shoots had formed.

Shoots produced on root cuttings did not respond as rapidly or at the high percentages of the seedling explants, but the explants did respond in culture. Within 8 weeks, axillary shoot elongation occurred in nearly half of the explants from shoots produced on root cuttings. The explant response indicates that shoots produced on root cuttings may be an alternative explant source for tissue culture studies.

Discoloration of the medium was observed for all explant sources. Explant exudation caused a reddish-brown discoloration of the medium at the basal end of the explants. Explant exudation has been documented in other members of the Annonaceae and was attributed to phenolics and polyphenoloxidase in the medium (Jordan et al., 1993). Tissue damage can cause explant exudation of phenolic compounds that turn brown as oxidation occurs (Hartmann et al., 1997).

This study indicates that tissue culture of pawpaw is possible, but the ontological age of the explants must be considered. Explants from mature sources require an extended period of time to induce explant responses. This study provides preliminary information about the effect of age on establishment of pawpaw in culture, but additional research is necessary to develop a micropropagation system for the pawpaw.

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