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Somatic Embryogenesis in White Oak (*Quercus alba*)©

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INTRODUCTION

White oak (*Quercus alba*) is an important forestry species. The implementation of forest fire controls in the 20th century have resulted in a major decline of native stands of white oak (Abrams, 2000). Further complicating oak survival is their potential susceptibility to sudden oak death (*Phytophthora ramorum*). Propagated by seed in the nursery industry, white oak availability is limited due to nursery production difficulties. There is a need for a clonal propagation system for selection of desirable characteristics such as fall color, hardiness, pathogen resistance, and improved nursery production characteristics.

White oak seed can be sown immediately after collection without any special treatments. This method of propagation however does not provide superior cultivars to the nursery industry. A clonal in vitro system of propagation produced from superior mature clones could result in increased profits for both liner and field production of white oak. In addition, somatic embryogenesis has proven to be a useful tool for recovering transgenic plants. This could be an important component in a strategy to develop plants resistant to diseases such as sudden oak death. However, the development of complete somatic embryogenesis systems has generally been difficult in oaks (Wilhelm, 2000).

In 2005, a single staminate catkin explant produced a somatic embryogenic culture. A low frequency of somatic embryogenesis from male catkins has been previously reported in other oak species (Gingas, 1989 and Wilhelm, 2000). This culture has continued to produce secondary somatic embryos for the past year. Therefore, the specific objectives of the current study were to evaluate the impact of stage of catkin development and growth regulator treatment on somatic embryo induction and to attempt to convert secondary somatic embryos from the 2005 culture into seedlings.

MATERIALS AND METHODS

Staminate catkins were collected three times during April and surface sterilized in 10% bleach for 15 minutes followed by a triple rinse in distilled water. The first collection (April 17) resulted in catkins less than 0.7 cm. Samples this small would not allow for the removal of male flowers and the entire staminate catkin was used. The final two collections (April 20 and 24) resulted in fully expanded catkins prior to anther dehiscence. Male flowers were removed from half of the staminate catkins. At each collection, five explants were placed per Petri dish on MS media (Murashige and Skoog, 1962), containing 1 or 5 µM 2,4-dichlorphenoxyacetic acid (2,4-D) plus 1 µM benzlyadenine (BA) or 5 µM naphthalene acetic acid (NAA) plus 1 µM BA. Explants were cultured in the dark or under cool white fluorescent lamps (PAR 60 $\mu mol \cdot sec^{-1} \cdot m^{-2}$) at 21 ℃. There were ten replicate Petri dishes per treatment for the first collection and five replicate dishes per treatment for subsequent collections. The percentage of explants forming callus was evaluated after 1 month.

A single staminate explant from 2005 formed somatic embryos. It entered a cycle of repeated secondary embryo formation. In May 2006, individual somatic embryos

Table 1. The percentage of staminate catkin explants with stamens removed forming callus after treatment with placed on a medium with 1 or 5 μM 2,4-dichlorphenoxyacetic acid (2,4-D) plus 1 μM benzlyadenine (BA) or 5 μM naphthalene acetic acid (NAA) plus 1 μM BA under light or dark conditions.

BA [1μM] media plus	Light	Dark
2,4-D [1μM]	43	100
2,4-D [5μM]	66	83
NAA [1μM]	0	33

Table 2. Somatic embryo development in white oak secondary somatic embryos derived from mature anthers placed on several conversion media.

	Stage of somatic embryo development			
Conversion media	Globular	Cotyledon	Germinating	Secondary embryos
Untreated	33.3a[z]	29.2a	0	37.5c
GA_3 [5 μM]	8.3b	16.7b	16.7a	58.3b
BA [1 μM]	0c	4.2c	0	95.8a
BA [1 μM] + ABA [5 μM]	29.2a	4.2c	0	66.7b

[z] Means followed by the same letter within a column were not different at $P \leq 0.05$ by Tukey's HSD test.

(globular stage) were moved either to basal MS medium or media containing 5 μM gibberellic acid (GA_3) alone, or 1μM BA plus 1 or 5 μM abscisic acid (ABA). There were six explants per Petri dish and four dishes per treatment. Explants were cultured as previously described. Germination and progression to cotyledon stage embryos without secondary embryo formation was evaluated after one month.

RESULTS AND DISCUSSION

The loss due to fungal contamination was 64% or greater. Removal of male flowers significantly decreased the percentage of contamination and increased the percentage of callus formation. Catkins from the first collection (April 7) failed to make callus, while those from the second two collections responded similarly. Therefore, data in Table 1 was for explants from the second two harvest dates that were not contaminated and had the flowers removed.

After 1 month, explants treated with 2,4-D formed a higher percentage of callus than those treated with NAA (Table 1). Callus developed along the peduncle on 2,4-D media, while the slight appearance of callus growth on NAA cultures occurred only at the site of removal from the mother plant and did not spread along the length of the peduncle as observed with 2,4-D treated explants.

Cultures placed in the dark produced more callus than light grown cultures. Dark grown cultures also produced a more friable callus without any green pigmentation compared to light grown cultures where the callus was denser with islands of green pigmentation. At present, no cultures have become embryogenic.

Some development to the cotyledon stage of development was observed on the growth regulator free medium, but the greatest percentage of secondary embryos beginning to germinate was induced on the medium containing GA_3 (Table 2). A similar effect of GA_3 on somatic embryo development was previously observed in willow oak (*Q. phellos*) cultures derived from seedling explants (Wells et al., 2005). These encouraging data suggest that plantlets can be derived via somatic embryogenesis in white oak but additional work is needed to produce an efficient system to induce somatic embryos from staminate catkins that convert to viable plantlets.

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