Somatic Embryo Development in Willow Oak©

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INTRODUCTION

Willow oak *(Quercus phellos)* is an important landscape plant and forestry tree generally propagated by seed for commercial production. Recent propagation of willow oak has been through cuttings taken from juvenile stock plants; however this does not allow for selection of mature characteristics such as autumn color, tree shape, winter hardiness, or ease of production. Somatic embryogenesis would allow for the mature mother plant to be rejuvenated into a juvenile form for cutting propagation while still having the clonal characteristics desired (Geneve et al., 2003).

Somatic embryogenesis has been reported in a number of oak species, with the majority of the work being performed in English *(Q. robur)* and cork oak *(Q. suber)*. In these species, the frequency of somatic embryo induction is between 80% and 100% from immature zygotic embryo explants but less than 15% using seedling leaf tissue (Wilhelm, 2000). However, regardless of the initial source, somatic embryo maturation, conversion, and germination have been difficult. Often the somatic embryo forms shoots or roots only, and complete recovery of plants is at a low frequency (Wilhelm, 2000).

Typical treatments used to enhance normal somatic embryo formation and encourage conversion include abscisic acid (ABA) and altering the osmotic potential of the medium using sucrose, mannitol, and sorbitol. Treatments used to stimulate germination in oaks are cytokinins and gibberellic acid (Wilhelm, 2000). The objective of this research was to investigate the effects of ABA, cytokinin, gibberellic acid, and sucrose concentration on development of somatic embryos derived from immature cotyledons of willow oak.

MATERIALS AND METHODS

Acorns were collected in August and surface-sterilized in 10% bleach for 15 min, followed by a dip in 70% ethanol and rinsed three times with sterile water. Cotyledon halves from the zygotic embryo were placed on MS (Murashige and Skoog, 1962) basal media in Petri plates containing 1 pM benzyladenine (BA) and 0, 1, 5, or 10 µM naphthaleneacetic acid (NAA). These plates were then placed under cool white fluorescent lights (16-h lighted photoperiod, PAR 60 µmol. ^{s}ec-1^{m}-2) at 21 "C. Explants were transferred to MS medium containing no growth regulators every 3 weeks until somatic embryos formed.

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Table 1. Percentage of somatic embryos forming a root or shoot after 2 months on MS media containing combinations of sucrose with abscisic acid or gibberellic acid.

Growth regulator	Concentration (µM)	Sucrose concentration (%)	
		3	6
ABA	0	15%	6%
	1	4%	18%
	5	7%	0%
GA_3	10	6%	16%
	50	20%	24%

Figure 1. Secondary somatic embryo formation in oak after 3 months.

Somatic embryos that reached the cotyledon stage were moved to media containing ABA (0, 1, or 5 µM), GA3 (0, 10, or 50 µM), or BA (0, 1, or 10 µM) in combination with 30 or 60 Oi of sucrose. Shoot and root development were evaluated after 2 months.

RESULTS

Somatic embryos formed at all concentrations of BA and NAA evaluated with the greatest percentage being produced at 5 µM NAA (45%). Those at 10 µM NAA produced somatic embryos at 11% and there was no difference between 1 0M NAA and the control (4%).

The use of ABA or GA, only slightly increased the number of somatic embryos producing a root or a shoot (Table 1). On average there was no difference between the two concentrations of sucrose. However, the highest frequency was seen using 50 µM GA3 and 6% sucrose. Including BA in the media had no effect on shoot or root production (data not shown).

Somatic embryos producing either a root or shoot were more frequent than the development of a seedling producing both. Seedlings having both a radicle and a shoot were transferred into a perlite and peat potting mix under high humidity, but none of the seedlings developed into plantlets.

DISCUSSION

NAA was effective at inducing somatic embryos in willow oak. NAA is often more effective than 2,4-D at inducing somatic embryogenesis in various oak species (Wilhelm, 2000). An auxin source was important in inducing primary somatic embryogenesis in willow oak, but secondary somatic embryos formed readily and repeatedly on basal medium without growth regulators (Fig. 1).

ABA is often used during somatic embryogenesis to promote more normal embryo development, but ABA usually inhibits embryo germination. Therefore, it was unexpected that ABA would promote shoot and root growth (Table 1). In cork oak, ABA reduced the development of new secondary embryos (Bueno et al., 1992). It is possible that by suppressing secondary somatic embryo formation, ABA allowed the continued development of the primary embryo that allowed it to germinate.

Gibberellic acid can be used to promote germination in slowly developing somatic embryos. Previous work with other oak species showed that GA_3 had a minimal effect at promoting somatic embryo germination (Kim et al., 1994; Ishii et al., 1999; Sanchez et al., 2003). More often, BA has been shown to stimulate shoot and root growth in oak (Wilhelm, 2000). However, in willow oak BA was ineffective at promoting germination, while GA_3 was as effective as ABA (Table 1).

Doubling the sucrose concentration did not consistently impact somatic embryo development or germination, but there was a trend towards a higher frequency of embryos with roots or shoots when grown at 6% sucrose (Table 1). Sucrose plays the dual role of providing a carbohydrate source for growth and acting as an osmoticum. It is possible that the sucrose concentration used in this work was not high enough to impact embryo development. Using cork oak, Garcia-Martin et al. (2001) found that 150 of sucrose allowed 75% of the somatic embryos to convert to seedlings. This conversion rate is comparable to the improvement in conversion of English oak to 83% found by slowly drying somatic embryos for 3 weeks prior to germination (Wilhelm, 2000).

To date, no plantlets have been recovered from willow oak via somatic embryos. Future research will focus on adjusting the water potential of the somatic embryo by drying or exposure to high osmotic concentrations to promote more normal seedling development.

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