Somatic Embryogenesis and Callus Induction in Willow Oak©

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INTRODUCTION

Oaks (*Quercus* sp.) are important nursery and forestry species. Seed is used to propagate most oaks because they are difficult to root from cuttings and many oaks experience delayed graft incompatibility. This severely limits availability of superior cultivars for the nursery trade. The ability to propagate superior mature clones of oak would result in increased selection and therefore, profitability for oak liner and shade tree production. It would also allow growers to put existing oak cultivars on their own roots rather than attempting to graft these cultivars (i.e., *Q. palustris* 'Crown Right'). In addition, development of the proposed somatic embryogenesis system would provide an appropriate system for attempts to transform mature oaks with novel genes (i.e., any potential genes developed for disease resistance to oak wilt or bacterial leaf scorch).

Although oaks are considered difficult to root from cuttings, it has been demonstrated that juvenile cuttings of oak root easily (Drew and Dirr, 1989). There have been numerous attempts to manipulate the ability of oaks to root from cuttings by using etiolation (Zaczek et al., 1999), grafting mature scions on to seedling understocks (Zaczek, 1999), rooting epicormic shoots (Harmer, 1988; Wang and Rouse, 1989), and mound layering (Griffin and Bassuk, 1996). These studies demonstrate that rooting in oaks can be enhanced if mature stock plants are subjected to rejuvenation. Currently, willow oak cultivars are being commercially propagated from cuttings obtained from juvenile stock plants. This demonstrates the commercial potential but these cultivars were seedling selected rather than selected from mature hardy plants.

The objective of this research is to develop a clonal system for propagation of mature oaks by rejuvenation using a step-wise process that includes: (1) inducing somatic embryogenesis from mature acorns from which the ovules have been removed; (2) creating juvenile stock plants from germinated somatic embryos; and (3) rooting cuttings from these juvenile stock plants.

MATERIALS AND METHODS

Acorn pieces from willow oak (*Q. phellos*) were collected in mid-summer after normal ovule abortion. Disinfested acorn halves with the viable ovule removed (dates 5 Aug. and 15 Aug.) or the embryo alone (dates 15 Aug. and 21 Aug.) were placed on a combination of 2, 4–dichlorphenoxyacetic acid (2, 4–D) or naphthalene acetic acid (NAA) at 1, 5, and 10 μM plus benzlyadenine (BA) at 1 μM for 15 days before being moved to growth regulator-free medium for expression of somatic embryogenesis. Explants were cultured in Petri dishes containing MS medium (Murashige and Skoog, 1962) under cool white fluorescent lamps (PAR 60 $\mu Mol \cdot sec^{-1} \cdot m^{-2}$) at 21 ºC.

Greenhouse-grown seedling willow oaks were produced in flats containing Metromix 350 for 2 or 4 months. Softwood cuttings were treated with an IBA quick dip (0, 5,000, and 10,000 ppm) and rooted under intermittent mist with bottom heat in the greenhouse. The percentage of rooted cuttings and the average number of roots per rooted cutting were evaluated after 30 days.

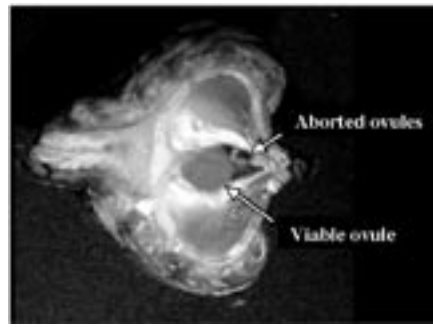

Figure 1. Viable and aborted ovules in 15-month-old willow oak.

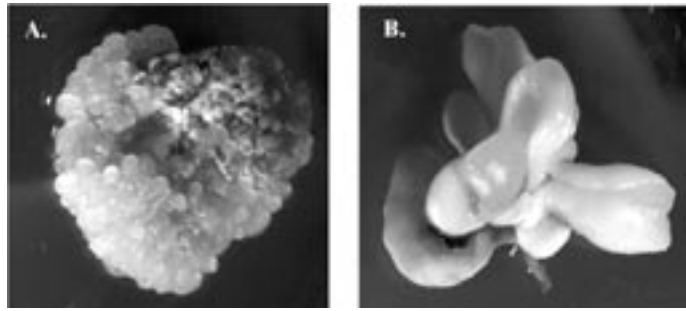

Figure 2. Callus and somatic embryogenesis in willow oak. A. Callus production after 6 weeks in acorn tissue treated with 5 μM 2, 4-D plus 1 μM BA. B. Somatic embryo production from zygotic embryo explants treated with 10 μM NAA plus 1 μM BA.

RESULTS

In willow oak, pollination occurs in early spring, but fertilization of the ovule is not completed until 15 months later. There are five ovules per acorn but only one usually remains viable in the mature fruit (Fig. 1). The tissue between the outer fruit wall (pericarp) and the ovule is diploid female in origin. It is not clear if it is fruit (mesocarp) or nucellar.

Callus growth was achieved from acorn pieces treated with 5 mM 2,4–D plus 1 μM BA (Fig. 2a). Callus has continued to proliferate, but to date no somatic embryos have formed. Embryo explants produced somatic embryos when treated with 5 or 10 μM NAA plus 1 μM BA (Fig.2b).

Cuttings taken from 2- or 4-month-old stock plants rooted at high percentages when treated with 5000 or 10,000 ppm IBA (Table 1). Roots per rooted cuttings increased with 10,000 ppm IBA.

DISCUSSION

Somatic embryogenesis has been achieved in a number of oak species from either embryo or vegetative tissue (Cuenca et al., 1999). Most of the species evaluated to date

Table 1. Adventitious rooting in greenhouse-grown seedling stock plants of willow oak.

IBA [ppm]	Two-month-old stock plants		Four-month-old stock plants	
	Rooting (%)	Roots per rooted cutting	Rooting (%)	Roots per rooted cutting
0	37.5b[z]	1.9c	43.4b	2.6c
5,000	64.2a	3.3b	73.9a	5.8b
10,000	70.8a	9.2a	78.3a	9.5a

[z]Means within a column with the same letter were not different P≤0.05 by LSD.

are not hardy northern oaks, except for a preliminary report in *Q. rubra* (Rancillac et al., 1996) using seedling leaves and a study in *Q. bicolor* (Gingas, 1991). The report with *Q. bicolor* is especially important because it involved somatic embryo formation from male catkins. Recently, Merkle and Battle (2000) using sweet gum (*Liquidambar*), have also demonstrated that flower tissue has a high capacity to form somatic embryos. Regeneration from flower parts represents a clonal form of regeneration from mature tissue, rather than the more typical "embryo cloning" found in somatic embryogenesis from zygotic seedling tissue reported for most woody plants.

Somatic embryogenesis would create a complete reversion from a mature state to a juvenile state as is achieved during normal zygotic embryogenesis (Hartmann et al., 2002). Somatic embryos derived from diploid female tissue (acorn sections) after removal of the ovules would be clonal. Therefore, if somatic embryogenesis is achieved from acorn-derived callus, the resultant plantlets would form juvenile stock plants suitable for cutting propagation. Work is ongoing to this end.

LITERATURE CITED

Cuenca, B., M.C. San-Jose, M.T. Martinez, A. Ballester, and **A.M. Vieitez.** 1999. Somatic embryogenesis from stem and leaf explants of *Quercus robur* L. Plant Cell Rept. 18:538-543.

Drew, J.J. and **M.A. Dirr.** 1989. Propagation of *Quercus* L. species by cuttings. J. Environ. Hort. 7:115-117.

Gingis, V.M. 1991. Asexual embryogenesis and plant regeneration from male catkins of *Quercus*. HortScience 26:1217-1219.

Griffin J. and **N. Bassuk.** 1996. Preliminary progress on the asexual propagation of oaks. Comb. Proc. Intl. Plant Prop. Soc. 46:487-494.

Harmer, R. 1988. Production and use of epicormic shoots for the vegetative propagation of mature oak. Forestry 61:305-316.

Hartmann, H.T., D.E. Kester, F.T. Davies, Jr., and **R.L. Geneve.** 2002. Hartmann and Kester's Plant propagation — principles and practice, 7th ed. Prentice Hall, Upper Saddle River, New Jersey.

Merkle, S.A. and **P.J. Battle.** 2000. Enhancement of embryogenic culture initiation from tissues of mature sweet gum trees. Plant Cell Rept. 19:268-273.

Murashige, T. and **F. Skoog.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Rancillac, M., A. Klinguer, S. Klinguer, and **B. Millet**. 1996. Preliminary investigations on somatic embryogenesis from leaf discs of red oak (*Quercus rubra* L.). Plant Growth Reg.. 20:67-73.

Wang, Y.T. and **R.E. Rouse.** 1989. Rooting live oak rhizomic shoots. HortScience 24:1043.

Zaczek, J.J. 1999. Micro-positional differences in cutting origin influence propagation of *Quercus rubra*. Comb. Proc. Intl. Plant Prop. Soc. 49:361-368.

Zaczek, J.J., C.W. Heuser, Jr., and **K.C. Steiner.** 1999. Low irradiance during rooting improves propagation of oak and maple taxa. J. Environ. Hort. 17:130-133.