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Original article

Axillary bud proliferation of 2 North American oak species: *Quercus alba* and *Quercus rubra*

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Summary — *Quercus alba,* white oak, and *Quercus rubra,* northern red oak, were selected to develop *in vitro* plantlet regeneration methods from bud and embryo explants. Various hormonal combinations were applied to explants to induce axillary bud proliferation. Maximal multiple shoot production was obtained when an intermediate micromolar range of benzyladenine (0.44–4.44 μM) was applied alone or in combination with low concentrations of naphthaleneacetic acid (1.0–100 μM). *In vitro* rooting of 1 *Q alba* microshoot was accomplished.

axillary bud proliferation / *Quercus* / *in vitro* / regeneration

Résumé — Prolifération de bourgeons axillaires de 2 chênes nord-américains, *Quercus alba* et *Quercus rubra.* *Des méthodes de multiplication* in vitro *à partir de bourgeons ou d'embryons ont été développées. De nombreuses combinaisons hormonales ont été testées pour induire la prolifération de bourgeons axillaires chez les explants. Les meilleurs résultats ont été obtenus avec une solution micromolaire de benzyl adénine variant de 0,44 μM à 4,44 μM appliquée seule ou en mélange avec de l'acide naphtalèneacétique (1,0–100 μM). L'enracinement* in vitro *de microplants de* Q alba *a été obtenu.*

prolifération de bourgeons axillaires* / Quercus / in vitro / *régénération

INTRODUCTION

The genus *Quercus* contains some of the most commercially important hardwood species in the world. In North America, *Quercus alba* L, white oak, and *Quercus rubra* L, northern red oak, are the 2 most valuable oak species used by the lumber and furniture industries. Veneer quality trees command a premium price and are a significant commodity in the wood export market.

Clonal propagation of valuable trees is being explored in a number of species. The genetic fidelity of clones make them of potential value for a variety of purposes, ranging from research on genotype x environmental interaction to increasing com-

mercial yields. *Quercus* species, however, have had a relatively small role in clonal forestry because they are difficult to vegetatively propagate. Two commonly used sources of explant material, rescued embryos and buds, both terminal and lateral, from young seedlings, were used to develop *in vitro* axillary bud proliferation systems for *Q alba* and *Q rubra*.

MATERIALS AND METHODS

Plant materials

Quercus rubra acorns were obtained from bulked collection made in the Shawnee National Forest in southern Indiana, USA, and stratified for 90 days prior to embryo removal. Seedlings of *Q alba* were from bulked acorns obtained from the Chuck Swan State Forest in eastern Tennessee and grown for 3 months before buds were harvested.

Sterilization procedures

The outer seed coat and subsequent tissue of *Q rubra* acorns were removed to expose the cotyledons, followed by immersion in a 20% commercial bleach (Clorox)/H_2O, v/v, plus 0.2% Tween 20 solution for 5 min. The remaining tissue was further dissected to remove 90% of the cotyledons to produce a rectangular block containing the embryonic axis. The tissue block was sterilized for 2 min in a Clorox solution (as above) and rinsed 3 times in sterile water for 5 min each, followed by removal of the remaining cotyledonary tissue to isolate the embryonic axis for *in vitro* culture.

Greenhouse-grown *Q alba* seedlings were treated with a fungicide spray (Benomyl, at label application rates) once-a-week after emergence from soil. At age 3 months, the seedlings were harvested at ground level and placed in a solution of Zyban (2.5 g of 75% WP/L H_2O), a broad spectrum systemic-contact fungicide for 24–36 h under fluorescent light and a moderate air flow to promote rapid transpirational uptake of the fungicide solution. After the fungicide soak, the leaves were removed, and the stem axis was immersed in a 70% ethanol/water, v/v, dip for 45 s and rinsed in sterile H_2O for 2–3 min. The stems were then placed in a 20% Clorox/H_2O, v/v, plus 0.2% Tween 20 solution for 4 min and rinsed in sterile H_2O for 5 min. Under sterile conditions, a dissection microscope was used to aid removal of the outer bud scales, followed by excision of the buds. The buds were placed in a 5% Clorox/H_2O solution for 5 min, given 3 rinses in sterile, distilled H_2O and placed into culture tubes.

Culture medium

The mineral medium (VSV) developed by Vieitez *et al* (1985) for *in vitro* regeneration of *Quercus robur* L was selected as the basal medium and was modified to contain various combinations of 2 plant growth regulators, benzyladenine (BA) and naphthaleneacetic acid (NAA).

Quercus rubra *embryo culture*

A single experiment was conducted using VSV medium containing different combinations of BA (44.4 nM, and 0.44, 4.44 or 44.4 μM) and NAA (0, 1.0 nM, 10 μM) in a Latin square design with a minimum of 10 replications/treatment. The embryos were kept on hormone medium for 22 weeks, when data were recorded. Cultures were transferred to fresh medium every 6 weeks.

Quercus alba *bud culture*

Experiments were conducted using VSV medium containing different combinations of growth hormones in Latin square designs. Experiment 1 was a preliminary study to investigate if *in vitro* bud elongation was possible and used the same concentrations of BA and NAA as in the *Q rubra* study. Experiments 2, 3 and 4 used BA concentrations as above, with various NAA levels. Experiment 2 used NAA levels of 0, 1.0 nM, 100 nM and 1.0 μM, while experiments 3 and 4 used NAA levels of 0, 1.0 nM and 1.0 μM. The number of explant buds in each treatment varied

among experiments, ranging from 5 to 12 buds. Buds were kept on hormone medium for 12, 6, 23 and 12 weeks in experiments 1, 2, 3 and 4, respectively.

Observations

All cultures were scored for shoot development and proliferation. The cultures were also observed for callusing.

RESULTS

Quercus rubra *embryo culture*

All embryos produced callus growth, although callusing was very limited in treatments without NAA. The extent of callus production increased with higher NAA concentrations. Maximal multiple shoot production with respect to number of explants/treatment and number of shoots/explant was in the 4.44 μM BA/O NAA treatment. Sixty percent of the explants in that treatment produced multiple shoots with a maximum of 6 shoots/embryo. Addition of NAA to 4.44 μM BA reduced both the number of explants producing shoots and the number of shoots/explant.

Quercus alba *bud culture*

An initial experiment demonstrated that buds could be induced to elongate in culture. Explants in treatments involving the combinations of 4.44 μM BA and 0 or 1 nM NAA resulted in the highest percentages of shoot development, 72% and 100% respectively. Maximum callus production occurred with the 44.4 μM BA and 10 μM NAA treatment combination. Based on these results, the maximum level of NAA used in experiments 2–4 was 1.0 μM, with an intermediate level (100 nM) between 1.0 nM and 1.0 μM added in experiment 2.

The results of experiments 2, 3 and 4 each showed that the highest percentages of explants producing multiple shoots occurred in treatments whose BA levels were 0.44 nM or 4.44 μM and NAA concentrations ranged between 0 and 100 nM. Experiment 3 (23 wk on hormone medium) induced the most shoots/explant (40), but had fewer explants producing multiple shoots than experiments 2 and 4.

One explant in this series of experiments produced 17 shoots. These shoots were excised and transferred to separate culture tubes to promote further development. Subsequently, 1 microshoot rooted spontaneously in culture, producing 1 rapidly elongating root. Transfer of this regenerate to soil under greenhouse conditions was unsuccessful.

DISCUSSION

The results showed that axillary buds on individual explants of *Q alba* (fig 1) and *Q rubra* could be induced to elongate and form multiple shoots *in vitro.* Multiple shoot production was optimal when an intermediate micromolar range of benzyladenine (0.44–4.44 μM) was used alone or in combination with low concentrations of napthaleneacetic acid (1.0–100 nM). The length of time on hormone medium appeared to have a positive effect on the number of multiple shoots produced and a negative effect on the percent of explants that responded. *In vitro* regeneration of one *Q alba* plantlet indicated that axillary-bud proliferation has potential for use in micropropagation.

The limited success of these experiments provides justification for future studies aimed at the micropropagation of these species. Unfortunately, all cultures were

Fig 1. Multiple shoot production by *Quercus alba* terminal (a) and lateral (b) bud explants *in vitro.*

lost to contamination, internal or external, or gradually lost vigor and died. Episodic growth in culture was judged to be a significant factor in the cultures' demise. Despite exposing the cultures to continuous light or long photoperiods the explants continued to exhibit dormancy cycles. Initiation of a new growing period reduced overall vigor and was eventually followed by explant death. Success of micropropagation systems in these *Quercus* species may depend upon the efficiency of generating multiple shoots within a particular growth phase.

REFERENCE

Vietiez AM, San-José MC, Vieitez E (1985) *In vitro* plantlet regeneration from juvenile and mature *Quercus robur* L. *J Hortic Sci* 60, 99-106