IN VITRO PROPAGATION OF PIN OAK

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Abstract.--Conditions were defined for in vitro propagation of pin oak (Quercus palustris Muenchh.). Competence of seedling explants averaged 93% on agar media compared to 41% in liquid culture; however, variable numbers (19-52%) of agar-produced shoots developed abnormally whereas normal shoot development occurred in liquid culture. Decreasing benzylaminopurine (BAP) from 8.9 to 0.089 µM in agar media did not affect competency but decreased the frequency of abnormal shoots and halved the yield of primary shoots. Shoots were subcultured in liquid modified Schenck and Hildebrandt or broadleaf tree media with 0.89 pM BAP. Eighty percent or more of the shoots could be rooted after indole-3-butyric acid treatment, but only plantlets rooted on filter paper bridges were successfully established in potting mix. Plantlets were acclimatized to greenhouse conditions.

Keywords: Quercus palustris Muenchh., micropropagation, tissue culture.

INTRODUCTION

Regeneration of trees and establishment of selected clones are necessary steps it various tissue culture strategies are to be effectively used in forest tree improvement programs. Notable success has been achieved with a few species in which trees have been regenerated from individual protoplasts, and clones of genetically transformed trees have ben developed (Haissig et al., 1987). However, for many important forest trees, such as oaks, suitable conditions for simple aicropropagation from explants have not been defined.

Limited research on the improvement of oaks has revealed broad variation among individual tree in heritable growth traits (Cech, 1971: LaFarge and Lewis, 1987). The ability to clone select individuals would have obvious advantages, but traditional methods of grafting and rooting of cuttings have generally proved to be inefficient or unreliable for most species of oak.

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To overcome these problems, method for tissue culture propagation have been applied with success to several Quercus spp. Several species have been micropropagated from seedling explants, and at least two species from explants taken from mature trees (Bennett and Davies, 1986; Meier-Dinkel, 1987; San-Jose et al., 1988).

Pin oak (Quercus palustris Muenchh.) is a major forest tree species on wet sites and heavy clay soils in the East and Midwest, particularly along the Ohio and Mississippi River drainages (Fowells, 1965). Although of limited timber value, it is a fast growing tree important for mast production in waterfowl habitat and is one of the most valuable ornamental oaks in the United States (McArdle and Santamour, 1987).

Lineberger (1980) reported callus development for pin oak but found no development of roots or shoots in culture. Bennett (1986) stated that pin oak could be micropropagated by methods developed for shumard oak (Bennett and Davies, 1986) but no details were given. In this paper we present results of research done to define parameters for in vitro propagation of pin oak and establishment of the derived plantlets.

MATERIALS AND METHODS

Seedling explants were taken from 4- to 12-week-old pin oak seedlings grown from stratified acorns sown in a commercial peatlite mix fertilized with Osmocote (17:7:12) and Micromax Plus (Sierra Chem. Co.). Seedlings were grown under a 16-hr photoperiod in a greenhouse. Stems were cleaned under running tap water, dipped in 95% ethanol for 30-90 sec and submerged in l.0% Na0Cl containing 0.04 Tween 20 for 20 min. After three rinses in sterile distilled water, single-node explants about 2.5 cm long were cut from the surface sterilized stems and placed in culture.

To obtain explants from mature trees, dormant branch terminals were cut during February-April and forced in a greenhouse under a 16-hr photoperiod. The branch terminals were kept in a solution of streptomycin sulfate (50 mg/l) and benomyl (10 mg/l a.i.) to discourage growth of bacteria and fungi. As new softwood shoots were produced they were harvested, surface sterilized as described above and cut into explants each with two or more nodes.

Explants were placed in 150 X 25-mm tubes containing either 14 or 3 ml 3f agar medium (0.6% w/v Difco Bacto agar) or 3 ml of liquid medium (Bennett and Davies, 1986). Broadleaf tree (BTM) (Chalupa, 1983), a modified Schenck and Hildebrandt (MSH) (Vieitez et al., 1985) and woody plant (Lloyd and McCown, 1980) media were used; all contained 3% w/v sucrose and were adjusted to pH 5.3. Media were supplemented with growth regulators as indicated prior to autoclaving. Explant cultures were placed under cool-white fluorescent lighting (40-70 µE/sec/m2) with a 16-hr photoperiod at 26°C. Explants were transferred to fresh media after 2 days, and thereafter, 3 ml liquid and agar cultures were transferred weekly and 14 ml agar cultures every 3 wk.

Shoot subculturing and most research on rooting were done in GA-7 vessels (Magenta Corp.) containing 20 ml of liquid medium and four or five

explants or subcultured shoots. Temperature and lighting were as described above, and media were changed biweekly. In the rooting phase, three or four. IBA-treated shoots were placed on filter paper bridges over 20 ml of medium in GA-7 vessels.

Secondary and tertiary shoots from subcultures were used in rooting experiments. Shoots were treated with indole-3-butyric acid (IBA) by (1) incorporating the IBA (3.7µM) into half-strength MSH with 3% sucrose and 0.6% agar,(2) dipping the shoots in 2.5 mM IBA for 15 min (Bennett and Davies), 1986) before culturing them on the same medium without IBA, or (3) a modification of the trea ment method of Zimmerman and Fordham (1985). In the latter method, three or four shoots were placed in tubes with 1.0 ml of half-strength MSH or BTM containing 3% sucrose and various concentrations of IBA. Individual shoots were transferred after 5 days to the same medium containing 0.6% agar without IBA, or to filter paper bridges over half-strength MSH or. BTM with 3% sucrose and no hormones.

Plantlets were placed in pots containing a vermiculite and perlite mix (1:1 v/v) and watered with half-strength BTM salts. Potted plantlets *were* placed in flats over a tray of water and covered with clear plastic propagation domes. Plantlets were kept under 60 µE/sec/m2 of mixed cool-white and Sylvania GroLux lamps with a 14-hr photoperiod at 23° C. Each week the plastic domes were changed with ones containing an increasing number (6, 12, 24, or 48) of equally spared 6-mm diameter holes to gradually reduce the humidity to ambient levels. Plantlets were watered as needed with sterile, half-strength BTM salts until well established and placed in a greenhouse.

RESULTS AND DISCUSSION

Shoot Production and Subculture

Composi tion of the medium and the presence of agar affected explant competence and the quality of primary shoots. Competency was more than twice as great on ag r medium than in liquid culture (Table 1). However, shoot quality was often poor on agar media. Fifty percent or more of the primary shoots were abnormal with thick stems, unexpanded leaves and necrotic stipules. Prima-y shoots produced in liquid culture had thin, often red-pigmented stems and expanded leaves with no evidence of necrosis (Fig.1). Two to three primary shoots were produced per competent explant in 4 wk. (Table 1).

We found that the average number o primary shoots produced was not a sufficient way to compare the effects **O;** medium composition on production of quality shoots. Many small shoots <1.0 cm long died when excised for subculture or rooting or failed to elongate when dominant shoots were removed from the explants. Therefore, a weighted shoot index based on shoot lengths and numbers was calculated for each competent explant (Table 1). Average shoot index was greatest for primary shoots produced on explants on BTM with agar. This medium also produced the fewest abnormal shoots.

Table 1. Effects of three tissue culture media and the presence of agar on primary shoot production by pin oak explants.

Mediaz	% Competent Explants	Growth parameters for competent explantsY: No. of shoots	Shoot indexx	% Abnormal shoots
Agar (0.6%)				
Broadleaf tree	96	3.2a	6.3a	19
Woody plant	100	2.6a	4.8b	52
Modified Schenck & Hildebrandt	84	1.8a	3.1c	29
Liquid				
Broadleaf tree	42	1.6a	2.9a	0
Woody plant	37	2.3a	4.0a	0
Modified Schenck & Hildebrandt	44	1.9a	3.2a	0

z All media contain 3% sucrose, 8.9 uM BAP, pH 5.3.

Y Mean separation in columns for agar or liquid media by Duncan's multiple range test, P = 5%. Growth parameters rated at 4 wk. N = 30-44.

x Shoot index per explant = shoot length factor x no. of shoots. Shoot length factors and corresponding shoot lengths were: 1 = 0.5 - 0.9 cm; 2 = 1.0 - 1.9 cm; 3 = 2.0 - 2.9 cm; 4 = 3.0 - 3.9 cm; 5 = 4.0 cm and >.

Several experiments were done to determime the effects of benzyl-aminopurine (BAP) concentration and presence of agar on primary shoot production. When BAP concentrations of 8.9, 0.89 and 0.089 uM were compared in BTM agar culture (14 ml, 0.6% agar), explant compentency was unafffected, and the percent abnormal shoots tended to decrease with decreasing BAP concentration. The trend in shoot quality was quite variable among several experiments on agar BTM and may be related to other factors such as physiological age and condition of the initial explants. Mean number of shoots per explant (about 2.4) was the same for 8.9 and 0.89 μ_M BAP but decreased to 1.1 primary shoots per explant at 0.089 μM BAP. Shoot index was greater at 0.89 µM BAP than at 8.9 or 0.089 uM within an experiment, but the absolute values for shoot indices tended to vary among experiments. When 3 ml liquid cultures (BTM, 8.9 M BAP) were compared to 3 ml agar cultures on the same medium, the only difference was in explant competence; 22% in liquid and 100% in agar cultures. No abnormal shoots occurred in either 3 ml liquid or 3 ml agar cultures and primary shoots numbers (2.4) and shoot indices (4.4) were not significantly different. It appears that both BAP concentration and presence of agar can effect primary shoot numbers and quality, and that agar in some way strongly affects explant competence. The varying effects of agar or other gel matrices on growth parameters in tissue culture have been described, but the cause(s) are not easily explained (Chun et al., 1986; Lee et al., 1986).

Pin oak shoots could be subcultured easily in liquid medium (BTM or MSH) in GA-7 vessels. A BAP concentration of 0.89 µM was found best because nigher concentrations caused the production of many buds and short shoots that did not develop further when subcultured. Subcultured primary shoots produced an average of 1.7 secondary shoots from axillary buds after 2 to 3 weeks. Initial explants with primary shoots removed produced about three new primary shoots after 2 weeks and produced two or three additional flushes over 4 to 6 weeks before they declined and died. Competency of shoots and initial explants ranged from 81 to 93% in liquid subculture. Abnormal shoot development (Fig.1) did not occur in liqid subculture, and abnormal primary shoots from agar culture produced normal secondary shoots from axillary buds after transfer to liquid subculture. Pin oak shoots have been carried successfully through four subcultures in liquid medium with production of rootable shoots.

Results over three years suggest that it will be possible to establish in vitro shoot cultures with explants From mature trees. Softwood shoots forced from dormant branches yielded explants with < 20% contamination. Explant competence on agar ranged from 0-50%, depended on the source tree and varied from year to year from the same tree. The results suggest that research on auxin/cytokinin ratio and time of sampling during the dormant season are needed.

In Vitro Rooting

Subcultured pin oak shoots were rooted successfully by all three IBA treatments. There was no significant difference (P = 0.05) in percent rooting (44-62%) or in number of roots per shoot (3 or 4) after 3 wk between the dip treatment or placing excised shoots on agar medium containing IBA. A post-treatment dark period of up to 2 wk was not beneficial to rooting. Toxicity symptoms often occured after the dip treatment. Treatment by the method modified from Zimmerman and Fordham (1985) gave similar rooting percentages and roots per shoot as the other treatments after 3 wk on agar medium, and 70-90% rooting has been obtained consistently 6-7 wk after treatment. IBA concentrations of 25, 75 or 123 µM gave no sifnificant differences in rooting percent. Numerous attempts to transfer rooted shoots from agar media o various potting mixes were unsuccessful. Examination showed that the -roots lacked root hairs which probably prevented plantlet establishment.

Vigorous root systems with abundant root hairs were produced when shoots treated with 123 M IBA by the modified Zimmerman method were placed on filter paper bridges over liquid medium (Fig.2) . Eighty percent or more of :he shoots rooted within 2 to 3 weeks.

Several attempts to root IBA-treated shoots by the method described by Bennett and Davies (1986) with Jiffy-7 pellets (Jiffy Prod. Ltd.) or ver-iculite were unsuccessful. Shoots became necrotic within 2 wk after IBA treatment, and root development did not occur.

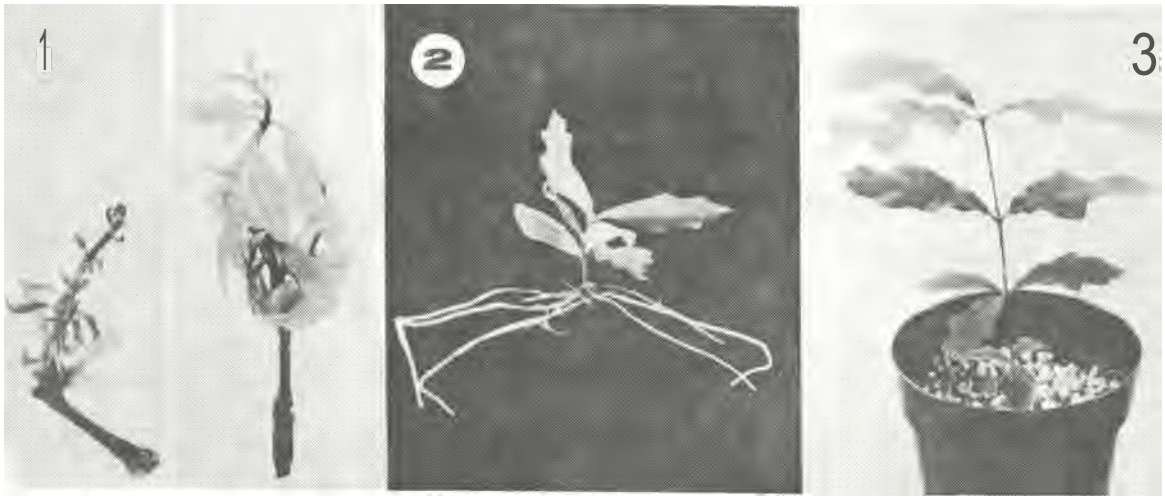

Fig. 1. Primary shoots produced after 4 wk in culture. Abnormal shoot from agar culture (left), normal shoot from liquid culture (right).
Fig. 2. Pin oak plantlet rooted on a filter paper bridge over half-strength BTM
Fig. 3. In vitro propagated pin oak acclimatized to greenhouse conditions.

Acclimatization

In repeated trials, 80 to 93% of rooted plantlets were successfully adapted to greenhouse conditions by the procedures described. During the 5- to 6-wk acclimatization period most plantlets produced two or three new growth flushes (Fig.3). No morphological differences have been noticed between micropropagated plants and seedlings grown from acorns.

SUMMARY

Pin oak was in vitro propagated from seedling explants. Broadleaf tree medium gave the best results of those tested. Agar greatly affected explant competence, and both agar and BAP affected shoot quality. Although an optimum concentration was not determined, 0.89 - 8.9 µM BAP in agar medium would strike a balance between high competence and good shoot quality. Shoot subcultures were maintained in liquid medium with 0.89 µM BAP. Subultures yielded high quality shoots and appeared to be a convenient way to clonally propagate pin oak. Several procedures for IBA treatment were adequate for

root initiation but good root hair development, as occurred on filter paper bridges, was required for plantlet establishment. Plantlets were readily acclimatized to greenhouse conditions if potted in a well-drained medium.

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