Acta Bot. Croat. 45, 55—61, 1986.

CODEN: ABCRA2
YU ISSN 0365—0588

UDC 582.632.2:578.085.23 = 20

IN VITRO GROWTH AND DEVELOPMENT OF OAKS (*QUERCUS ROBUR* AND *Q. PETRAEA*)*

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Received March 18, 1986

Experiments were carried out to establish an initial culture of *Quercus robur* and *Q. petraea*. Apical and axillary buds, taken from seedlings and young plants (up to two years), were used as initial explants for the establishment of culture. The buds were satisfactorily disinfected by successive use of chlorine products and mercuric chloride. Three nutrient media were used: mod. De Fossard medium, ACM medium and mod. WPM medium. Various concentrations of hormones BA, KIN, 2iP, NAA, IBA and GA_3 were added to all media investigated. Elongation of cultured axillary and apical buds and in some cases adventitious bud induction were achieved when ACM and mod. WPM media were used. Age of explants used did not have an inhibitory effect on positive reaction obtained on both media.

Introduction

Over the past years numerous studies on in vitro propagation of forest trees have been carried out. These investigations indicate that micropropagation may be a very good solution for a rapid propagation of superior forest trees (Abbott, 1978). Also, by this method it is possible to propagate trees with certain properties, for example the resistance to some diseases or air polution, increased quality and production of wood, or rapid growth. This procedure of plant production can be carried out throughout the year in a limited space and for a short period of time. Plants propagated in this way are usually relatively free of pathogens.

* This paper is dedicated to Prof. Z. Devidé on the occasion of his 65th birthday.

Oaks play a very important role among the forest species in the world and also in Yugoslavia, but until today very little has been done with the genus *Quercus* (Bellarosa, 1981, Chalupa, 1981, 1983, 1984, Jacquiot, 1975a, 1975b, 1976, Pardos, 1981, Vieitez et al., 1985). Therefore, in vitro culture investigations of *Quercus robur* L. and *Quercus petraea* (Mattuschka) Liebl. were planned and carried out.

In this report results obtained from the primary culture establishment of *Quercus robur* and *Q. petraea* are described.

Material and Methods

Material

Shoot apices from seedlings (10- and 20-day-old) grown in vitro and nodal stem segments and shoot apices from the young plants (1-, 2-, 4.5-, 12-, 16- and 23-month-old) grown from acorns in the greenhouse of *Quercus robur* and *Q. petraea* were used as initial explants. These were 10—20 mm long and all of them carried one small uninjured bud wrapped in scales.

Methods

Disinfestation of plant material was successively carried out with a 1% (w/v) water solution of Izosan-G (Pliva — Zagreb) for 20 min and then, after a sterile water rinse, with a 1% (w/v) mercuric chloride for 10 min. The buds were then washed with a sterile water solution of calcium chloride ($CaCl_2 \cdot 6\ H_2O$, 4.8 gl^{-1}) followed by four washes in sterile distilled water, each one lasting three min.

Single explants were inoculated on an agar nutrient medium in test tubes (23 × 160 or 30 × 120 mm), capped with cotton plugs and aluminium foil. The following three media: mod. De Fossard medium (Badia, 1982), Aspen Culture Medium, ACM (Ahuja, 1983) without lysine and mod. Woody Plant Medium, WPM (McCown and Lloyd, 1981) without chlorine ions and with 60 mgl^{-1} NaFeEDTA were used in experiments. Sucrose (2%) and Bacto-agar (0.9%) were added to all the media. Various concentrations and combinations of 6-benzylaminopurine, BA (0.1—2.2 μM), kinetin, KIN (0.4—2.3 μM), (2-isopentenyl)adenine, 2iP (0.5 μM), α-naphthaleneacetic acid, NAA (0.05 μM), indole-3-butyric acid IBA (2.5—5.0 μM) and gibberellic acid, GA_3 (0.3 μM) were used according to the experimental objectives. The pH was adjusted to 5.8 before autoclaving. All the media and growth substances used had beeen autoclaved at 1.2 $kgcm^{-2}$ and 120 °C for 15 min.

The cultures were kept in a temperature-controlled room at 24 °C ± 2 °C under the illumination of fluorescent lamps (TEŽ — Zagreb, 40 W, with a special range of 400—700 nm, 17 Wm^{-2}) and a light-dark cycle of 16—8 hours.

Results

The process of disinfestation with mercuric chloride was very satisfactory. The percent of sterile cultures was from 55—100% with *Quercus robur* and 40—100% with *Q. petraea*, depending on initial explants age.

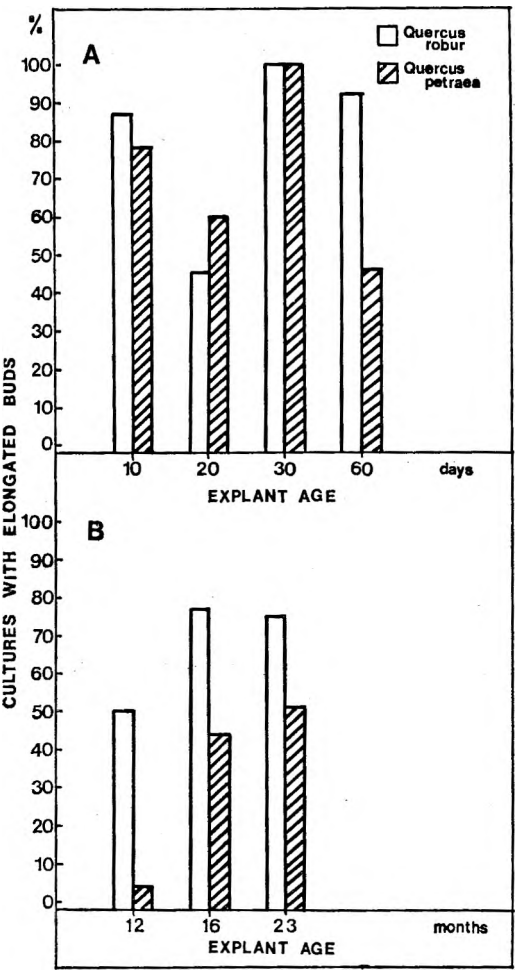

Fig. 1. Influence of explant age on bud elongation in primary culture of *Quercus robur* and *Q. petraea* (estimated after 6 weeks); **A**, Inducation medium ACM plus 0.1 μM BA, 0.5 μM KIN and 0.5 μM 2iP, explants used from 10- to 60-day-old donor plants; **B**, Induction medium mod. WPM plus 2.2 μM BA, 2.5 μM IBA and 0,3 μM GA_3, explants used from one- to two-year-old plants.

Elongation of buds was better in *Quercus robur* cultures than in *Q. petraea* (Figs. 1 and 3A) when mod. WPM and mod. De Fossard medium were used. However, the ACM medium was equally good for both species. Although on ACM medium with several combinations of hormones (BA, KIN 2iP and NAA) the percent of elongated buds was the greatest for both of investigated species (Table 1), we found mod. WPM medium with 2.2 μM BA, 2.5 μM IBA and 0.3 μM GA_3 more convenient. On that

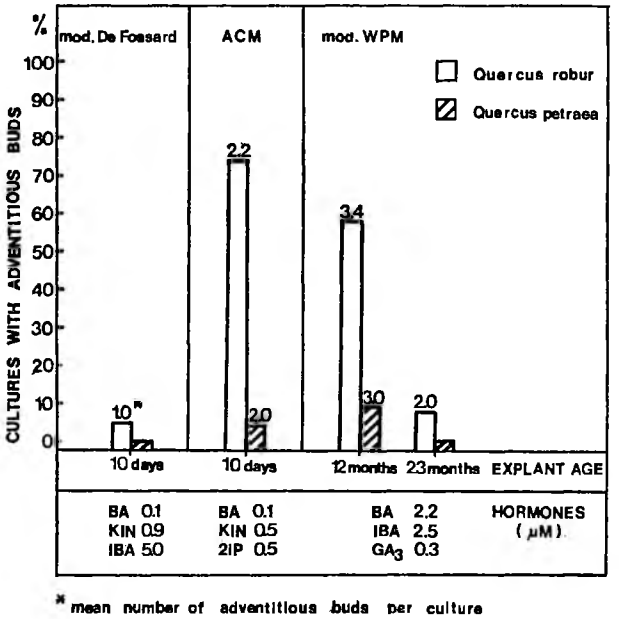

Fig. 2. Adventitious bud induction on primary internodal explants of *Quercus robur* and *Q. petraea* (estimated after 6 weeks in culture). Each treatment consisted of 24 or more replicates.

medium the shoots developed more vigorously, deeply green, never vitrificated and without polyphenols extractions in substrate. On the mod. De Fossard medium satisfied results were only obtained with the following combination of growth regulators (μM): 0.1 BA, 0.5 KIN and 0.5 2iP (Table 1). In general, De Fossard medium was not convenient.

Lateral shoots developed in 9% cultures of *Quercus petraea* (Fig. 3B) on ACM medium with 0.1 μM BA, 0.5 μM KIN and 0.5 μM 2iP, but never in *Q. robur* cultures.

Fig. 3. Regeneration in oak *Quercus robur* and *Q. petraea)* primary culture. **A,** Elongated shoots on *Quercus petraea* explant on mod. WPM with 1.3 μM BA, 0.5 μM KIN and 0.5 μM 2iP (x2.5); **B,** Lateral shoots developed in culture of *Quercus petraea* on ACM supplemented with 1.3 μM BA, 0.5 μM KIN and 0.5 μM 2iP (x3); **C,** Adventitious buds generated on explant base of *Quercus robur*, mod. WPM with 1.3 μM BA, 0.5 μM KIN and 0.5 μM 2iP (x3); **D,** Adventitious root developed in callus of *Quercus petraea* primary culture on mod. De Fossard medium with 1.3 μM BA, 0.9 μM KIN and 4.3 μM IBA (x3.5). Pictures taken after 6 weeks in culture.

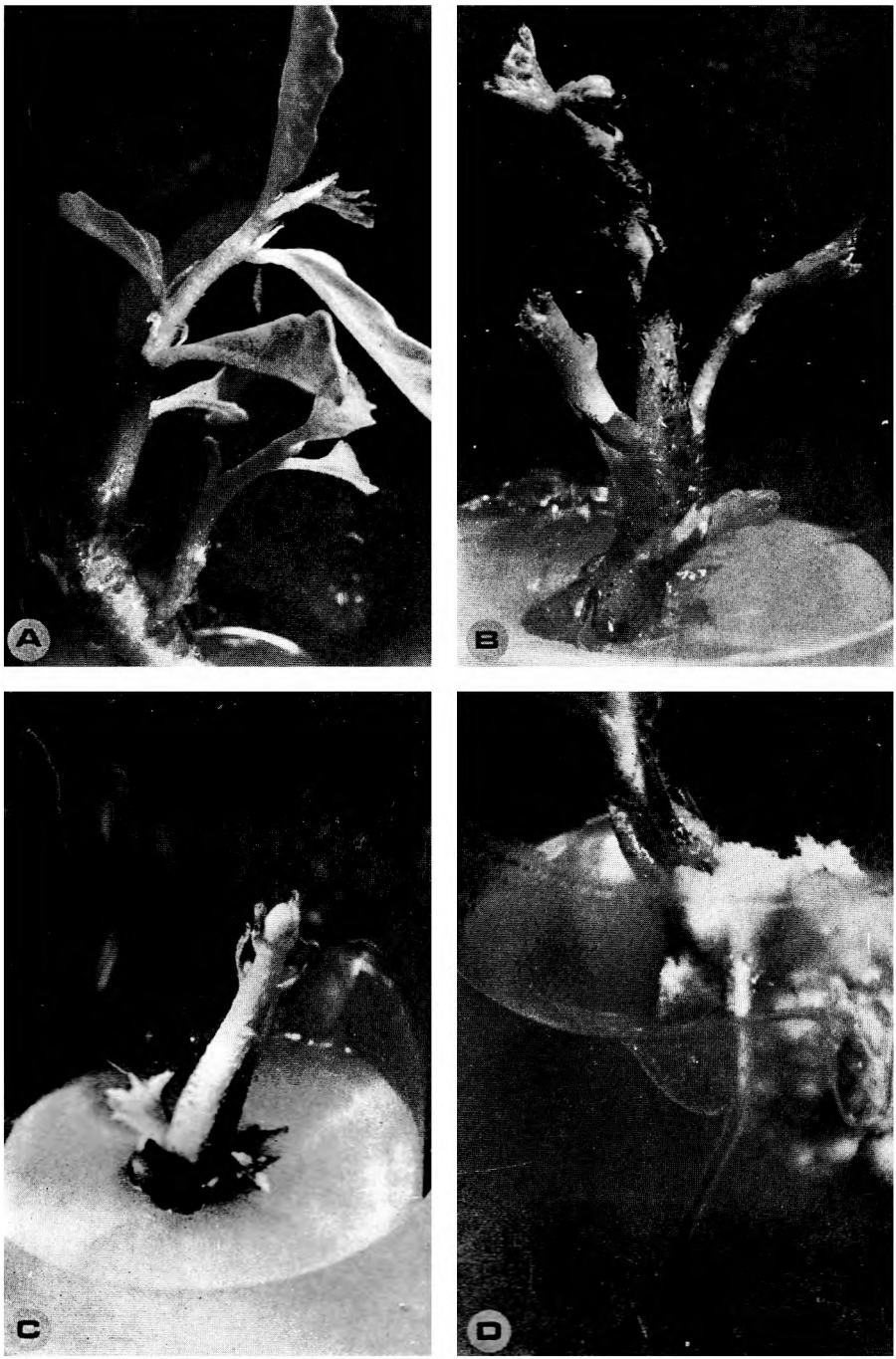

Fig. 3.

Table 1. Effect of basal medium and growth regulators on bud elongation (0.5—2 cm) of *Quercus robur* and *Q. petraea* in primary culture (estimated after 6 weeks).

MEDIUM	HORMONES (μM)						CULTURES WITH ELONGATED BUDS (%)	
	BA	KIN	2iP	IBA	NAA	GA_3	*Q. robur*	*Q. petraea*
mod. De Fossard	0.0	0.0	0.0	0.0	0.0	0.0	8	0
	0.1	0.5	0.5	0.0	0.0	0.0	88	21
	0.1	0.9	0.0	5.0	0.0	0.0	44	21
	2.2	0.0	0.0	0.0	0.0	0.0	0	7
ACM	0.0	0.0	0.0	0.0	0.0	0.0	40	50
	0.1	0.5	0.5	0.0	0.0	0.0	44	46
	0.1	0.5	0.5	0.0	0.05	0.0	32	21
	0.1	2.3	0.5	0.0	0.0	0.0	100	—
	0.4	0.4	0.5	0.0	0.0	0.0	100	—
	0.4	0.4	0.5	0.0	0.05	0.0	—	100
	0.9	0.9	0.5	0.0	0.0	0.0	100	100
	0.9	0.9	0.5	0.0	0.05	0.0	100	100
	2.2	1.4	0.5	0.0	0.0	0.0	33	—
	1.8	0.0	0.0	0.0	0.0	0.0	0	—
	2.2	0.0	0.0	0.0	0.0	0.0	100	100
mod. WPM	2.2	0.0	0.0	2.5	0.0	0.3	68	33

Adventitious buds were induced more frequently in *Quercus robur* cultures than in *Q. petraea* (Fig. 2 and 3C). On ACM medium with 0.1 μM BA, 0.5 μM KIN and 0.5 μM 2iP adventitious buds regenerated in 74% of *Q. robur* cultures with a mean number 2.2 buds and only in 4% cultures of *Q. petraea* ($\bar{x} = 2.0$). On mod. De Fossard medium adventitious bud induction was very low (Fig. 2). Better adventitious bud induction was obtained on the explants of younger donor plants.

Rarely, adventitious roots developed in culture of *Quercus petraea* when mod. De Fossard medium with 1.3 μM BA, 0.5 μM KIN and 4.3 μM IBA was used (Fig. 3D). Roots regenerated from callus developed on the cut surface of explants.

Discussion

Some types of woody plants still present a problem in establishing cultures, e.g. *Juglans*, *Castanea* or *Quercus* (Zimmerman, 1985).

Recently there have been a few reports on the possibility of in-vitro-multiplication of oak shoots (Chalupa, 1983, 1984, Vieitez et al. 1985). However, results are still incomplete and will have to be continually investigated.

On the base of our investigations on the different mineral salt composition and combinations of growth regulators, it seems that the composition of salts is as important as the adequate used hormones for the successful oak tissue culture. In our experiments the best elongation of apical buds and the release of axillary buds were achieved on the ACM and mod. WPM media with both investigated species. The dormancy release of axillary buds was satisfactory and there was no difference

between apical and axillary buds reactions. On the other hand, Pardos (1981) suggested in *Quercus suber* cultures that the explant position on the stem influenced the response. Chalupa (1981, 1983, 1984) reported that the best media for the establishment of initial culture with *Quercus robur* and *Q. petraea* were WPM and BTM media, while Vieitez et al. (1985) maintained that Heller and Gresshoff and Doy medium were the most satisfactory for *Quercus robur* in their experiments. It seems that oak tissue culture is very sensitive to composition of salts and their ratio in medium, to which much more attention should be paid.

In our cultures we also achieved induction of adventitious buds on the cut surface. That phenomenon has been described also for *Quercus suber* (Bellarosa, 1981, Pardos, 1981).

Badia (1982) reported that vitamin E seems to be efficient as an antioxidizing agent against polyphenolic compounds issued from the tissues, but we have not had such experience. We found that on mod. WPM medium, when initial explant age was 12, 16 or 23 months, there was no polyphenol extractions.

Our results indicate that it is possible to establish the culture of *Quercus robur* and *Q. petraea* in vitro. In our future investigations we shall try to provide optimal conditions for micropropagation of both species.

This work was supported by the Research Council of the SR Croatia (SIZ IV).

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SAŽETAK

RAST I RAZVITAK U KULTURI HRASTOVA *LUŽNJAKA (QUERCUS ROBUR)* I KITNJAKA (*Q. PETRAEA*) IN VITRO

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Istraženi su uvjeti za uspješno uvođenje eksplantata hrasta lužnjaka *(Quercus robur)* i kitnjaka *(Q. petraea)* u kulturi in vitro.

Kao početni eksplantati korišteni su vršni i aksilarni pupovi klijanaca i mladih biljaka starih od 10 dana do dvije godine. Pupovi su uspješno sterilizirani klornim preparatom Izosan-G (1%) i živinim kloridom (1%). Ispitana je reakcija eksplantata na tri osnovne hranidbene podloge: mod. De Fossard, ACM i mod. WPM i nekoliko regulatora rastenja (BA, KIN, 2iP, NAA, IBA i GA_3) u različitim koncentracijama i kombinacijama. Najpogodniji medij za produžni rast i normalni razvoj izdanaka bio je mod. WPM s 2,2 μM BA, 2,5 μM IBA i 0,3 μM GA_3. Međutim, najveći postotak produljenih izdanaka dobiven je na mediju ACM uz dodatak BA (0,4—2,2 μM), KIN (0,4—0,9 μM), 2iP (0,5 μM) i NAA (0,05 μM). U kulturama je opaženo i zametanje adventivnih pupova na proksimalnoj strani eksplantata na sva tri ispitana medija. Kultura hrasta lužnjaka bila je uspješnija u odnosu na kulturu hrasta kitnjaka u većini usporednih pokusa.

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