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Original article

Establishment of explants from 200-year-old *Quercus petraea* in culture

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Summary — As part of the ongoing EC-Cost 87 Woody Plant Research Program, methods of establishing mature material of *Quercus petraea* (Mattuschka) Lieblein in culture were examined. Unopened buds taken from shoot tips were difficult to disinfect. Shoots produced from branch segments under mist established well in culture but increasing branch age and decreasing branch diameter reduced shoot production. Shoots produced from buds that had flushed inside transparent plastic bags, while still attached to the tree, established well in culture. Explants established in culture produced more shoots under light containing a high proportion of the red spectrum.

***Quercus petraea* / tissue culture / mature / light**

Résumé — Multiplication végétative de chêne sessile âgé de 200 ans. *Les méthodes de multiplication d'arbres adultes de* Quercus petraea *(Mattuschka) Lieblein ont été mises au point dans le cadre du groupe de recherches EC-Cost 87. Les bourgeons apicaux encore fermés sont difficiles à désinfecter. Les pousses produites à partir de branches sous* mist *peuvent être mises en culture dans de bonnes conditions; cependant la production de pousses décroît avec l'âge de la branche et le diamètre des pousses. Les pousses développées à partir de bourgeons ayant débourré dans des sacs en plastique, alors qu'elles sont toujours attachées à l'arbre, se maintiennent correctement en culture. Les explants mis en culture produisent plus de branches sous une lumière ayant un spectre riche dans le rouge.*

Quercus petraea / *culture de tissu / maturité / lumière*

INTRODUCTION

This paper describes 3 methods used to establish cultures from explants of mature *Quercus petraea* harvested from the Rostrevor oak forest (1 of only 3 natural oak forests left in Northern Ireland). The trees used in these experiments have been dated by dendochronology and are between 200 and 300 years old (Pilcher, 1976).

A standard method of establishing a woody plant in culture would be to sterilize buds and place them on a suitable medium. Subsequent proliferation of lateral shoots is the generally accepted method of propagation *in vitro* to maintain the genetic integrity of a clone (Jones, 1991). Other methods are compared to this basic system.

As part of the ongoing Cost 87 Woody Plant Research Program, the method of

Vermeer and Evers (1990) was repeated (trunk sections of mature oaks produced shoots under mist and these were micropropagated). Branches were used to determine the thinnest section which could be used to produce shoots, thus reducing the potential damage to the trees. In a further effort to reduce the size of explant required, establishment of explants directly from buds flushed on the tree (enclosed in plastic bags to reduce contamination) was attempted.

MATERIALS AND METHODS

A commercial disinfectant Domestos was used (10% sodium hypochlorite and 4% non-ionic surfactants and soap, Lever Brothers, Kingston-upon-Thames, Surrey, UK). After sterilization, the explants were rinsed 3 times in autoclaved water.

Explants were cultured in woody plant medium (Lloyd and McCowan, 1980), 7 g/1 oxoid agar, 30 g/1 sucrose, pH 5.6, 6-benzylamino purine (BAP) was initially at 2 mg/l, then 0.2 mg/l in subsequent 4-week subculture passages (30 ml/container). The medium was dispensed into transparent, high-impact, polystyrene, disposable, 170-cm^3 containers. The cultures were grown at 20 °C with a 16 h photoperiod supplied by either Gro-Lux Thorn EMI (A) or Gelbweiss white Osram (B) lamps or a combination of both.

The spectra are shown in figure 1.

Shoot multiplication was determined using the multiplication coefficient (MC) defined by San-José *et al* (1988).

Methods of establishment

1. Closed buds were collected in March 1991 from 3 trees. In the case of 1 tree, buds from a young branch were also included. The buds were sterilized in 20% Domestos for 20 min.

2. A young branch (retaining its leaves) and a mature branch (both growing from the same position on the tree) were harvested, cut into 40-cm segments and placed in a 50:50 grit: compost mix in a greenhouse, under mist, to induce shoot production. The resulting shoots were harvested and established in culture. The cambial ages of the base segments of the young and old branches were determined ac-

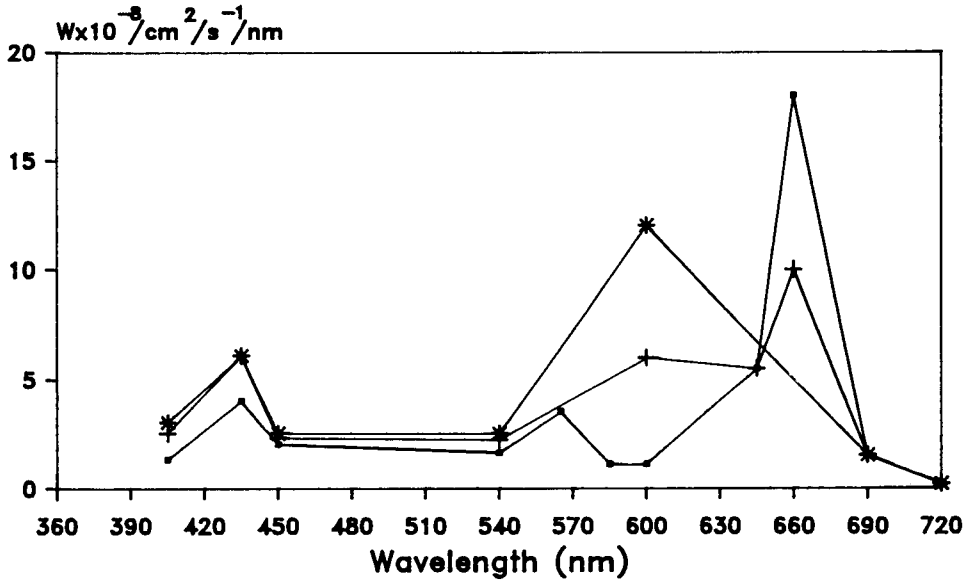

Fig 1. Light spectra at culture level. ■: Gro-Lux x 4; +: Gro-lux x 2 + white x 2; ✳: white x 4.

cording to their tree-ring patterns and found to be 23 and 41 years old, respectively. There was no replication.

3. While still on the tree, in March 1991, buds were dipped into pure ethanol for 3 min then enclosed in a transparent plastic bag which was taped onto the branch. One corner of the bag was cut off to allow ventilation. One month after flushing (April) these shoots and untreated controls were harvested and established in culture after sterilization in Domestos.

RESULTS

Method 1

Two out of 160 explants survived, both coming from the young branch of 1 tree. Losses were due to contamination. After 2 months in culture, the 2 surviving explants produced 12 shoots which were then placed under the different light regimes (4 shoots/light treatment). Shoot multiplication is shown in table I.

Method 2

Both the young and old branch segments produced a flush of shoots which were established in culture. Four flushes were obtained between April and July. No flush occurred during August.

Total shoot production (number of shoots x mean shoot length) for each segment is shown in table II. Shoot production declined with reduction in stem diameter.

No shoots were produced from the submerged parts of the segments. Shoot production occurred just below the cut surface of the segment and at the points from which side branches had arisen. Some segments from the young branch also produced shoots from points at which branching or sectioning had not occurred.

The older branch produced fewer shoots than the younger branch (table III).

Method 3

Buds which flushed inside the plastic bags produced longer shoots (mean 25 cm) than the controls (10 cm). Shoots harvested from inside the bags established in culture, whereas all of the explants from the control shoots were lost (table IV).

DISCUSSION

Of the 3 methods used, the least successful was method 1. Although establishment

Table I. The effect of light source on shoot number.

Light	*Number of shoots after each subculture*			
	0	*1*	*2*	*3*
4A	4	14 (3.5) [a]	27 (1.9)	95 (3.5)
2A + 2B	4	15 (3.8)	37 (2.5)	143 (3.9)
4B	4	6 (1.5)	12 (2.0)	32 (2.7)

[a] Multiplication coefficient between parentheses. See test for explanation of light levels.

Table II. The effect of stem diameter on shoot production (no of shoots x mean shoot length).

Cambial age (yr)	*Branch diameter (cm)*					
	1.0	*2.0*	*3.0*	*4.0*	*5.0*	*6.0*
23	0	66	162	55	231	741
41	0	7	105	183	0	25

Table III. The effect of branch age on shoot production (cm).

Cambial age (yr)	*Flush*			
	1st	*2nd*	*3rd*	*4th*
23	490 (65) [a]	393 (61)	245 (41)	123 (22)
41	128 (16)	75 (9)	228 (39)	111 (16)

[a] Total number of shoots per flush between parentheses.

Table IV. Effect of sterilization treatment and flushing in polythene on establishment in culture.

Domestos	*Explants (40/treatment)*	
	+ bag	*– bag*
60% 20 min	Dead	Dead
30% 20 min	43% healthy	Contaminated
15% 20 min	Contaminated	Contaminated

[a] Between June 1991 and July 1991, MC = 1.6. * Between July 1991 and August 1991, MC = 1.9.

was achieved from buds on a young branch (5%), no cultures were obtained from the old branch.

The second method showed that branches 3–6 cm in diameter could be used to produce shoots which could easily be established in culture, regardless of age. However, the difficulty of characterizing the origin of shoots produced from stem segments (Evers *et al*,1990) remains to be resolved.

Method 3 resulted in good establishment in culture from a smaller number of explants, with the advantage over method 2 of clear origin of shoots and minimal damage to the trees.

While table IV shows the effect of light on shoot multiplication for 1 clone of *Q petraea*, similar results have been obtained for *Q robur* (Evers, personal communication) *Q ilex*, *Q coccifera*, *Q conferta* and *Q agilops* (Kabrianis, personal communication). This method of propagation will have a major impact on the economics of micropropagation of oak for reforestation programs.

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