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Review article

Vegetative propagation of oak (*Quercus robur* and *Q petraea*) by cutting and tissue culture

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Summary —The potential of cuttings of *Quercus robur* and *Q petraea* to form adventitious roots decreased rapidly with increasing plant age. The rooting ability of older plants was increased by hedging. Hedging of stock plants offers an effective technique for the production of cuttings with high rooting potential. Stock plant environment markedly affected rooting of leafy cuttings. A high percentage of cuttings collected from plants grown under continuous light rooted. Vigorous plants were produced from cuttings which rooted quickly and were capable of rapid shoot growth immediately after rooting. Shoot growth of rooted cuttings was stimulated in suitable environmental conditions by sufficient mineral nutrition. Rooted cuttings which formed new long shoots and wintered in rooting medium in the same place in an unheated greenhouse exhibited high survival rates. For tissue culture propagation, 2 methods were used: micropropagation by axillary shoot multiplication and by somatic embryogenesis. Axillary shoot multiplication was stimulated on low salt media (BTM, or woody plant medium WPM) supplemented with a low concentration of benzylaminopurine (BAP) or N-benzyl -9-(2-tetrahydropyranyl) adenine (BPA) (0.2-0.6 mg•l^{-1}). Rooting of microshoots was achieved *in vitro* and was also successful under non-sterile conditions in a rooting mixture of peat and perlite. The field growth of micropropagated trees was comparable to that of control seedlings. Embryogenic cultures were initiated from immature zygotic embryos of *Q petraea* cultured on modified Schenk and Hildebrandt (SH) medium supplemented with BAP (1mg•l^{-1}). The majority of embryogenic cultures produced somatic embryos. The conversion of somatic embryos into plantlets was achieved after cold and desiccation treatment. Plantlets regenerated from somatic embryos were transplanted into potting mixture, where growth continued.

vegetative propagation / *Quercus* spp / cutting / tissue culture / somatic embryogenesis

Résumé — Multiplication végétative des chênes par méthodes horticoles et culture de tissu. *La potentialité des boutures de* Quercus robur *et* Q petraea *à former des racines décroît rapidement avec l'âge du pied mère. L'aptitude à l'enracinement d'arbres âgés est améliorée par une taille sévère du pied mère. Cette technique permet d'obtenir des boutures ayant une bonne aptitude à la rhizogenèse. Les conditions d'élevage des pieds mères ont une influence sur la production de racines des boutures feuillées. Les boutures prélevées sur des arbres élevés en lumière continue s'enracinent plus facilement. Des plants vigoureux peuvent être produits à partir de boutures s'enracinant rapidement et capables de croître en hauteur immédiatement après s'être enracinées. La croissance en hauteur des boutures est améliorée par une nutrition minérale adaptée. Les bou-*

tures enracinées ayant développé de nouvelles pousses et maintenues durant d'hiver dans leur milieu d'enracinement en serre non chauffée manifestent un taux de survie élevé. La multiplication végétative par culture in vitro *implique deux techniques : la multiplication de pousses axillaires et l'embryogenèse somatique. La production de pousses axilliaires est améliorée sur des milieux faiblement salins (BTM et WPM) et contenant de la BAP (ou BPA) en faible concentration (0,2–0,6 mg/l). L'enracinement de micropousses a été réalisé en conditions* in vitro *et en conditions non stériles sur des milieux constitués de tourbe et de perlite. La croissance au champ d'arbres issus de micropropagation est comparable à celle de semis. Les méthodes d'embryogenèse ont été réalisées à partir de culture d'embryons immatures de* Q petraea *faites en milieu SH additionné de BAP (1 mg/l). La majorité des cultures produisirent des embryons somatiques. La conversion des embryons en plants s'est faite à l'aide de traitements par le froid et la dessication. Ces plants ont été transférés en pot pour leur développement ultérieur.*

multiplication végétative / *Quercus* sp / bouture / culture de tissu / embryogenèse somatique

INTRODUCTION

Plants of oak species used for reforestation are traditionally raised from seed. The vegetative propagation of oak was considered difficult and has not been successful on a commercial scale. In many regions, good acorn harvests are not frequent and acorns are difficult to store. The vegetative propagation of oak may provide an adequate plant supply when there is a natural shortage of seeds and could reduce the demand for seed-grown planting stock, especially during years following poor seed harvests.

The increasing interest in vegetative propagation of oak over the last decade stimulated detailed studies, and new techniques have been developed which enable production of clonal plants either by a stem-cutting system or by *in vitro* methods. Vegetative propagation is important for oak tree improvement. The long reproductive cycle of oak is a serious obstacle to effective tree improvement by conventional tree-breeding techniques. Vegetative propagation is an important method for preserving the unique characteristics of some trees. *In vitro* propagation of oak species can be used for the production of plants with desirable genetic traits. Effective plant regeneration from meristems and embryogenic cultures is a prerequisite for application of recombinant DNA technology to improvement of oak trees.

Experiments with vegetative propagation of oak by cuttings were started a long time ago. The rooting of various oak species proved to be difficult and the progress in vegetative propagation of oak has been slow. Propagation of juvenile cherrybark oak (*Q falcata*) by cuttings was reported by Farmer (1965) and later Cornu *et al* (1975, 1977), Kleinschmit *et al* (1975), Garbaye *et al* (1977), Chalupa (1980, 1982, 1990a) and Spethmann (1982, 1985, 1986) described the production of rooted cuttings of important European oak species (*Q petraea* and *Q robur*).

Experiments with tissue culture propagation of oak started after trials with cuttings. Initially, efforts were focused on regeneration of plants from callus cultures. Callus formation was stimulated (Jacquiot, 1952; Seckinger, *et al* 1979; Srivastava and Steinhauer, 1982), however, plant propagation was not achieved. A system based on *in vitro* multiplication of shoots from axillary buds has been developed (Chalupa, 1979, 1981, 1983, 1984; Bellarosa, 1981; Pardos, 1981; Vieitez *et al*, 1985). Micropropagated plantlets were transplanted into soil and later were planted in the field. The system of axillary-shoot multiplication was used for micropropagation of various oak species: *Q robur* and *Q*

petraea (Chalupa, 1979, 1981, 1983, 1984, 1985, 1987b, 1988, 1990b; Vietez *et al* 1985; Pevalek-Kozlina and Jelaska 1986; Civinová and Sladky, 1987; Favre and Juncker, 1987; Meier-Dinkel, 1987; San-José *et al* 1988, 1990; Juncker and Favre, 1989; Volkaert *et al*, 1990), *Q suber* (Bellarosa, 1981, 1989; Pardos, 1981; Manzanera and Pardos, 1990), *Q Shumardii* (Bennett and Davies, 1986), *Q acutissima* (Ide and Yamamoto, 1986; Sato *et al*, 1987), *Q serrata* (Ide and Yamamoto, 1987) and *Q lobata* (Johnson and Walker, 1990).

Somatic embryogenesis has great potential to be used for mass clonal propagation of plants. Recently, somatic embryogenesis was induced in oak. Immature or mature embryos, anthers or seedling segments were used as the initial explants for induction of somatic embryogenesis in *Q robur* and *Q petraea* (Chalupa, 1985, 1987a, 1990c; Jörgensen, 1988), *Q suber* (El Maataoui and Espagnac, 1987), *Q acutissima* (Sasaki *et al*, 1988), *Q rubra* and *Q alba* (Gingas and Lineberger, 1989), Q ilex (Féraud-Keller and Espagnac, 1989), *Q cerris* (Ostrolucká and Pretová, 1991). Plant regeneration from oak somatic embryos proved to be difficult and the conversion of embryos into plants was achieved only in some species and at a low frequency.

In this report, results obtained in our experiments with vegetative propagation of *Q robur* and *Q petraea* by cuttings and by tissue culture are presented and discussed.

MATERIALS AND METHODS

Propagation by cuttings

Leafy softwood cuttings were used for rooting experiments with *Q robur* and *Q petraea*. Cuttings were collected from 6-year-old hedged stock plants (hedged 4–10 cm above the ground) and from seedlings and trees of different ages (1–30-yr-old trees). For each treatment, 40–90 cuttings were used. Cuttings were collected between May 20 and July 20. All cuttings were inserted into the rooting mixture 2–24 h after being taken from trees. Bases of leafy cuttings (10–20 cm long) were soaked in a hormonal solution (20–24 h in indole-3-butyric acid (IBA) 200 mg•1^{-1}) or treated with a talc-based rooting powder (1% IBA + 10% benomyl or 0.5% IBA + 0.1% naphthalene acetic acid (NAA) + 10% benomyl, and inserted into rooting mixture consisting of peat and perlite (1:1 or 1:1.5, v/v). Cuttings were rooted either under controlled environment (in growth cabinets equipped with a fog system) or in a greenhouse under an intermittent fog system. After rooting, relative air humidity and temperature were gradually reduced, and rooted cuttings wintered in the rooting mixture in the same place in the unheated greenhouse. Rooted cuttings were lifted the following spring (in early June, after formation of new shoots) and were transplanted in the nursery.

Propagation by tissue culture

Plant material

For initiation of *Q robur* and *Q petraea* organ cultures, explants were taken from shoots of seedlings 3–6-months-old. As the source of material from older trees, shoots or 6-year-old hedged trees, or stump sprouts (from stumps of 40-yr-old trees) were used. After removing all leaves, the axis was cut into shoot-tip and nodal segments 10–20 mm long, which were surface-sterilized in 0.1% mercuric chloride solution for 20–40 min. After 3 succesive rinses in sterile distilled water, the initial explants were placed on agar nutrient medium.

For initiation of somatic embryogenesis, immature seeds collected from 5 open-pollinated trees were used for experiments. Fruits were collected weekly in July and August. Seeds were surface-sterilized in calcium hypochlorite solution (7.5%, w/v) for 20 min and then washed twice with sterile distilled water. Immature embryos were excised from seeds and placed on agar nutrient medium. Explants (immature embryos, nodal segments) were cultured in 100 ml flasks containing 20 ml of nutrient medium. Each treatment involved 30–60 explants and was repeated twice.

Culture media and conditions

Organ cultures

Explants were cultured on modified Gresshoff–Doy (GD) medium (Gresshoff and Doy, 1972), BTM (Chalupa, 1984), or Woody plant medium (WPM) (Lloyd and McCown, 1980). The basal media were supplemented with glutamine (100 mg•l^{-1}). The media contained various concentrations (0.2-2.0 mg•l^{-1}) of the cytokinin (6-benzylaminopurine (BAP) or (*N*-benzyl-9-(2-tetrahydropyranyl)adenine (BPA). For rooting, NAA and IBA were used in concentrations ranging from 0.2 to 1.0 mg•l^{-1}. Difco Bacto agar (6 g•l^{-1}) was used to solidify nutrient media and sucrose (20 g•l^{-1}) as a carbon source. The media were adjusted to pH 5.7 before sterilization by autoclaving at 121°C for 20 min. Cultures were grown at 25°C in light with a 16-h photoperiod under cool white fluorescent lamps (60 uE•m^{-2} s^{-1}).

Somatic embryogenesis

Explants were cultured on modified Murashige–Skoog (MS) medium (Murashige and Skoog, 1962), Schenk–Hildebrandt (SH) medium (Schenk and Hildebrandt, 1972), and WPM (Lloyd and McCown, 1980), supplemented with glutamine (200 mg•l^{-1}) or casein hydrolysate (500 mg•l^{-1}). The media contained cytokinin BAP (0.2-2.0 mg•l^{-1}), and auxin (IBA 0.0-1.0 mg•l^{-1}, or 2,4-D 0.0-2.0 mg•l^{-1}). Media were solidified with Difco Bacto agar (6 g•l^{-1}). Sucrose was used as a carbon source (MS and SH medium 30 g•l^{-1} WPM: 20 g•l^{-1}). Cultures were grown at 25°C either in the dark or in light (16-h photoperiod or continuous light).

RESULTS

Vegetative propagation by cuttings

Rooting potential in relation to maturation and the effect of hedging

Vegetative propagation by cuttings is usually restricted to young material because aging reduce the ability to root cuttings. In *Q robur* and *Q petraea* the potential of cuttings to form adventitious roots decreased rapidly with increasing plant age. Cuttings taken from trees 1- and 3-year-old rooted at high frequencies and produced well-developed root systems. Cuttings from older trees (9-30-yr-old) rooted poorly (table I). Difficulties associated with aging make the direct use of cuttings from older trees unsuitable for rapid clonal propagation. The use of cuttings from young plants is limited because the quantity of cutting material which is produced by young ortet is low.

The rooting ability of older oak trees can be increased by cutting down the trees and by hedging stock plants. In our experiments, cutting down and hedging was effective in *Q robur* and *Q petraea*. Rooting potential of cuttings harvested from hedged 6-year-old plants of *Q robur* was high (table II). The stock plants were hedged every year and elongated sprouts were used for rooting. Hedging of oak stock plants offers an effective technique for the production of cuttings with high rooting potential and high survival.

Table I. Rooting of *Quercus robur* cuttings collected from plants of different ages.

Age (yr)	*Rooting (%)*	*Mean number of roots/ rooted cutting*
1	89a	3.8a
3	78a	3.2a
7	52b	2.6ab
9	39b	2.2b
20	18c	2.0b
30	11c	1.7b

The data are based on 50 cuttings/treatment. Values followed by the same letter are not significantly different at the 5% level.

Table II. The effect of hedging of *Quercus robur* (6-yr-old plants) on rooting of cuttings and on formation of new shoots during rooting.

Stock plants	*Rooting (%)*	*Mean number of roots/ rooted cutting*	*Formation of new shoots (%)*
Control plants	56a	2.7a	7a
Hedged plants	81b	3.9b	62b

The data are based on 90 cuttings per treatment. Figures followed by the same letter are not significantly different at the 5% level.

Effect of physiological condition of stock plant on rooting potential

Stock plant environment markedly affected rooting of harvested leafy cuttings. Irradiance, photoperiod and their interactions with nutrients had a marked effect on the rooting potential of leafy cuttings. In our studies, a long photoperiod (continuous light) improved rooting of *Q petraea* cuttings. Cuttings from seedlings grown under continuous light rooted in significantly higher percentages (92%) than those from seelings grown under natural daylength (76%).

Stimulation of shoot growth after rooting of cuttings

For successful vegetative propagation of oak, it is important not only to achieve rooting of cuttings, but to produce plants with low mortality and rapid growth. In our experiments with *Q robur*, cuttings which, after rooting, formed new shoots and had an active metabolic exchange between root system and stem, exhibited high survival rates. Vigorous plants were produced from cuttings which rooted quickly and were capable of rapid shoot growth immediately after rooting.

Cuttings harvested from hedged trees exhibited significantly higher frequencies of formation of new shoots than cuttings collected from intact control trees (table II). Shoot growth of rooted cuttings were also stimulated by mineral nutrition. Regular watering (every 2nd d) of rooted cuttings with diluted WPM (1/10 strength of macroelements) or incorporation of slow-release fertilizers into rooting mixture enhanced root quality and stimulated shoot growth. Supplemental nutrition with diluted WPM had a favorable influence on shoot elongation. The formation of new shoots was also stimulated by supplemental lighting. Cuttings grown under continuous light (cool white fluorescent lamps) formed new shoots at higher frequency (87%) than cuttings grown under a natural photoperiod.

Rooted cuttings, which formed new shoots and reached a total length of 30–50 cm in the autumn, wintered in the rooting mixture in the same place in an unheated greenhouse and suffered only small losses. The following spring, rooted cuttings were lifted (in early June) and transplanted in the nursery, where the growth continue. Their survival rate was high (78-94%) and vigorous plants were produced during the growing season.

Vegegative propagation by tissue culture

At present, two methods can be used for tissue culture propagation of oak: axillary shoot multiplication and somatic embryogenesis.

Micropropagation by axillary shoot multiplication

To establish cultures, we used actively growing shoots collected after bud flushing. Sterile nodal segments and shoot–tips of juvenile origin were placed on nutrient

medium and started to grow within 1–2 weeks. Among the media tested, the highest multiplication rate was obtained on low salt media (BTM, WPM) supplemented with a low concentration of cytokinin (BAP 0.2-0.6 mg•l^{-1}). Within 4–5 weeks, shoots elongated considerably and leaves developed. Explants grown on high salt media (MS, SH) produced short shoots.

The number of new shoots that were formed during the multiplication stage was moderated by cytokinin. Cytokinins BAP and BPA were the best stimulators of shoot proliferation of *Q petraea* and *Q robur*. The growth of axillary shoots was stimulated on WPM supplemented with a low concentration of BAP (0.2 mg•l^{-1}). Higher concentration of BAP (0.4-0.6 mg•l^{-1}) induced shoot proliferation and the number of produced shoots increased (table III). Shorter shoots were produced on medium containing a high concentration of BAP (2 mg•l^{-1}). The multiplication rate (number of segments usable for the next multiplication cycle) achieved on WPM supplemented with BAP was high (3–8, depending upon the clone).

Table III. The effect of cytokinins BAP and BPA on shoot proliferation of *Quercus petraea* (cultured for 5 wk on WPM).

Cytokinin (mg•l^{-1})	*Number of shoots*	*Shoot length (mm)*
BAP		
0.0	0.5a ± 0.4	7a ± 4
0.2	2.6b ± 1.2	19b ± 9
0.6	2.9b ± 1.4	19b ± 9
1.0	2.8b ± 1.3	17b ± 8
2.0	2.4b ± 1.1	15b ± 7
BPA		
0.0	0.5a ± 0.4	7a ± 4
0.2	2.8b ± 1.3	19b ± 9
0.6	3.2b ± 1.5	20b ± 9
1.0	2.9b ± 1.2	18b ± 8
2.0	2.4b ± 1.1	15b ± 6

The data are based on 7 juvenile clones and 3 subcultures (mean ± SD). Values followed by the same letter are not significantly different at the 5% level.

A new cytokinin, BPA effectively stimulated the formation of axillary buds and shoot proliferation. Tested clones of *Q petraea* produced more shoots on media containing BPA than on media supplemented with BAP. Many shoots were produced on WPM containing 0.6 mg•l^{-1} BPA (table III, fig 1).

Tissue culture propagation of adult trees was more difficult than propagation of seedlings. Shoots initiated at the base of the trunk retain juvenile characteristics and were used as the initial explants for the establishment of adult tree cultures (stump sprouts of 12 40-yr-old trees were used). The explants of adult trees were grown on the same media as seedling cultures. Explants from 7 trees produced multiplying cultures. The mean multiplication rate of cultures of adult origin was lower (by about 28%) than the rate of juvenile cultures, however, two genotypes exhibited the same proliferation rate as cultures of seedling origin.

Rooting of microshoots was achieved *in vitro* and was also successful under non-sterile conditions in rooting mixture. Agar media used for *in vitro* rooting contained no cytokinin and had a lower level of mineral salts. Cytokinins are strong inhibitors of adventitious rooting, and high-salt media had indirect inhibitory effects. GD agar media and WPM (half- or full-strength) containing a low concentration of auxin (IBA or NAA 0.2–1.0 mg•l^{-1}) stimulated root induction. Within 2–3 weeks, 68-92% of microshoots of juvenile origin (depending upon the clone) produced roots. Rooting percentages of microshoots initiated from adult trees were lower (by 24–78%, depending upon the clone), than those of microshoots of seedling origin.

Fig 1. Multiple axillary shoots of *Quercus petraea* produced from nodal segment cultured on WPM supplemented with 0.6 mg•l^{-1} BPA.

High rooting percentages of juvenile microshoots were also obtained by direct rooting in potting mixture. After auxin treatment (a quick dip of the microshoot base into liquid IBA, 1.0 g•l^{-1}, for 1 min), microshoots were inserted into potting mixture (peat and perlite, 1:1, v/v) and kept under a plastic sheet in a humid atmosphere. Mean rooting percentages of juvenile microshoots ranged from 54 to 80% (depending upon the clone). *Ex vitro* rooting was less laborious than *in vitro* rooting. Microshoot quality was very important in *ex vitro* rooting. Small microshoots (10–15 mm long) exhibited higher mortality rates. Fully developed leaves of microshoots were metabolically beneficial to rooting. Stem elongation and formation of new leaves stimulated adventitious root formation. The treatment of microshoots with rooting hormone was useful for increasing the speed and uniformity of rooting and the number of adventitious roots. For *ex vitro* rooting, humidity control was important. Shortly af-

ter adventitious root formation, active shoot growth resumed and the size of the plantlets increased substantially. The newly formed leaves were much less susceptible to desiccation. Plantlets were grown under high humidity for 5–8 weeks, then humidity was gradually reduced to normal levels. Plantlets grown under continuous light maintained shoot growth after root formation and exhibited higher survival rates.

After plantlets formed new adapted leaves on elongated shoots and reached the height of 10–20 cm, they were transferred outdoors and grown in partial shade for 2–3 months. Most rooted plantlets of juvenile origin survived (76–94%) and continued to grow. After hardening off, the plants were planted in the field, usually in early summer. Planted trees attained a height of 20–30 cm at the end of the second growing season. In the following years, the growth of micropropagated trees continued. Indeed there was no significant difference in growth between the micropropagated plants and control seedlings. At the end of the 8th growing season, the micropropagated trees were more than 230–290 cm high. The trees exhibited normal growth and appearance.

Plant regeneration by somatic embryogenesis

Somatic embryogenesis is a promising method of clonal oak multiplication. Our experiments showed the feasibility of using immature zygotic embryos for initiation of highly embryogenic tissue and formation of oak somatic embryos.

In our experiments with somatic embryogenesis in *Q petraea* embryogenic cultures were initiated from immature zygotic embryos cultured on modified SH and MS media and on WPM supplemented with cytokinin. Zygotic embryos excised from immature seeds collected in July and early August produced embryogenic tissue most frequently; 48–76% of cultured immature zygotic embryos produced embryogenic cultures (table IV). Embryogenic cultures were initiated on modified SH and MS media and WPM (containing 500 mg•l^{-1} of casein hydrolysate), supplemented with BAP (1 mg•l^{-1}) or BAP (1 mg•l^{-1}) plus IBA (1 mg•l^{-1}). The immature zygotic embryos cultured on these media produced embryogenic tissue within 7–9 weeks (fig 2). The embryogenic competence was maintained by embryogenic tissue subculture. Embryogenic tissues cultured on modified SH medium containing cytokinin kept their embryogenic potential for more than 3 years. Developing somatic embryos were often loosely attached to parent tissue. Secondary somatic embryogenesis was frequent. Adventitious embryos developed gradually into mature somatic embryos.

Somatic embryos conversion was achieved after alternations of physical conditions and medium changes. The conversion of somatic embryos into plantlets was

Table IV. Initiation of embryogenic tissues from cultured immature embryos of *Quercus petraea*.

Collection date	*SH medium*	*MS medium*
	% embryogenic cultures	
July 3	48	52
July 10	64	70
July 17	76	68
July 24	65	62
July 31	58	56
August 7	54	55

Explants were cultured on modified SH medium or MS medium containg BAP (1 mg•l^{-1}) and casein hydrolysate (500 mg•l^{-1}). For each treatment 70–80 immature embryos were used.

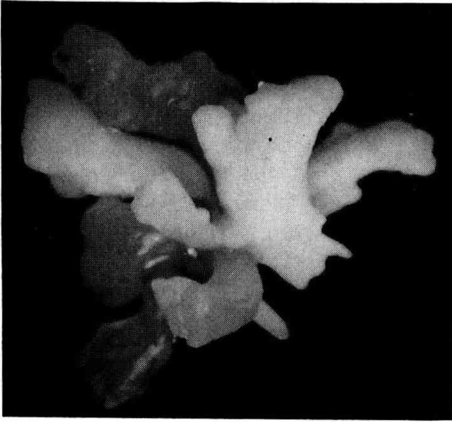

Fig 2. Embryogenic culture of *Quercus petraea* initiated on modified SH medium (containing casein hydrolysate 500 mg•l^{-1}), supplemented with BAP (1 mg•l^{-1}).

stimulated by exposure to cold (2–3 °C for 3–4 wk) and desiccation (dehydration of somatic embryos inside sterile sealed dishes for 2-3 wk). After desiccation, somatic embryos were transferred into WPM containing a low concentration of cytokinin (BAP 0.1 mg•l^{-1}) and were cultured under continuous light to induce conversion; 12–18% of embryogenic cultures produced germinating somatic embryos. Some somatic embryos produced only roots, some embryos produced shoots and roots (fig 3). The plantlets with growing shoots and roots were subcultured individually on WPM without cytokinin. More than 90 plantlets of *Q petraea* regenerated from somatic embryos were transplanted into potting mixture. Plantlets were grown under high air humidity and continuous light. After acclimatization, 62 plants of *Q petraea* regenerated from somatic embryos were planted in the nursery.

Fig 3. Conversion of a somatic embryo of *Quercus petraea* into a plantlet: after exposure to cold and desiccation somatic embryo was transferred on WPM and cultured under continuous light.

DISCUSSION

Vegetative propagation offers the opportunity to use valuable genotypes in commercial forestry. Vegetative propagation is an alternative to a breeding system based on seed orchards. It seems that seed orchards are difficult to use in breeding oaks due to their long reproductive cycle and low acorn production.

The problem of aging plays an important role in vegetative propagation (Bonga, 1982, 1987; Durzan, 1984, 1990). The idea to propagate mature-plus oak trees is not easily applicable. For successful clonal oak propagation, juvenile tissue is essential as the initial explant. Shoots originating from juvenile zones of the tree exhibit juvenile characteristics (Schaffalitzky de Muckadell, 1954, 1959). Experiments with various tree species (Bonga, 1982, 1987; Hartmann and Kester, 1983; Franclet *et al,* 1987) and our experiments with oaks indicate that cuttings made from stump sprouts and from hedged stock plants cut back every year are juvenile explants which root easily. Experiments show that cutting down and hedging of oak trees is an efficient method to obtain juvenile material from older trees.

For possible use of cuttings in commercial forestry, rooted cuttings with high survival rates and good growth and morphology must be produced. The physiological status of stock plants had great influence on rooting potential and mortality of rooted cuttings. Correct timing of cutting collection, sufficient mineral nutrition, a reliable fog system and effective irradiance during the rooting process favored the production of rooted cuttings with high survival rates. Rooting cuttings, which formed new shoots shortly after rooting and wintered in an unheated greenhouse, exhibited high survival and rapid shoot growth during the following growing season.

The importance of tissue culture as a propagation method of oak continues to grow. A system based on micropropagation by axillary shoots has been developed (Chalupa, 1979, 1981, 1983, 1984; Bellarosa, 1981; Pardos, 1981; Vieitez *et al,* 1985) and proved to be effective. Recently the system has been refined (Bennett and Davies, 1986; Meier-Dinkel, 1987; Chalupa, 1988, 1990b; San-José *et al,* 1988, 1990) and used for production of plants for field testing. Experiments indicate that tissue culture propagation of oak will become a useful tool for the clonal multiplication of selected plants. Plants produced from tissue cultures are as vigorous as plants produced by conventional methods. Field growth of micropropagated oak trees of juvenile origin was comparable to that of control seedlings. It is anticipated that the axillary-shoot multiplication method will continue to be the main tissue culture method for oak propagation.

Development of somatic embryogenesis as a propagation method continues and new information on initiation of embryogenic culture and oak regeneration has been published (Chalupa, 1987a, 1990c; Sasaki *et al,* 1988; Gingas and Lineberger, 1989). Experiments showed the feasibility of using immature embryos for initiation of highly embryogenic tissue and for formation of oak somatic embryos. *In vitro* induced embryogenesis often depended upon the presence of growth regulators in the nutrient medium, however, their role is not clear. Some species required the presence of auxin in medium for the induction of embryogenesis, for other species this substance was not essential. The main problem is the low frequency of conversion of oak somatic embryos into plantlets. Before somatic embryogenesis is used as a propagation method, many problems must be solved.

Currently available results and knowledge indicate that a stem-cutting system

and micropropagation by tissue culture are promising methods for clonal oak propagation. Close association of micropropagation and the stem-cutting techniques will perhaps enable the development of an integrated system to be used for mass propagation of selected oak clones; for example, micropropagation may provide the initial multiplication stage prior to stem-cutting propagation.

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