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Short Note: In Vitro Induction of Organogenesis in Juvenile and Mature Beech

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Abstract

Bud explants from three mature trees (more than 70 years old) and embryonal explants derived from the seeds of the same trees, belonging to three cultivars of European beech, *Fagus sylvatica* L., namely, 'Zlatia', 'Pendula', and var. *purpurea*, were cultured on modified Woody Plant Medium. Only bud explants from var. *purpurea* showed limited differentiation of shoots; bud explants from 'Zlatia' and 'Pendula' remained largely unresponsive under the experimental conditions. The embryonal explants from all three cultivars exhibited shoot formation, although the number of shoots was mostly limited to a few. Occasional plantlet regeneration from embryonal explants were observed.

Key words: European beech (*Fagus sylvatica*), embryonal explants, bud explants from mature trees, tissue culture, shoot differentiation, plantlets.

Zusammenfassung

Knospenexplantate von drei über 70jährigen Bäumen und Embryoexplantate von Samen dieser Bäume wurden auf einem modifizierten "Woody Plant Medium" kultiviert. Die Bäume gehören zu drei Kultivaren der europäischen Buche, *Fagus sylvatica* L., und zwar 'Zlatia', 'Pendula' und var. *purpurea*. Nur Knospenexplantate von var. *purpurea* zeigten eine begrenzte Differenzierung der Sprosse, die Knospenexplantate von 'Zlatia' und 'Pendula' zeigten im wesentlichen kein Wachstum. Dagegen zeigten die Embryoexplantate aller drei Kultivare Sproßdifferenzierung, obwohl jeweils nur wenige Sprosse gebildet wurden. In einzelnen Fällen konnten sich aus den Embryoexplantaten Pflänzchen bilden.

Introduction

Tissue explants from mature forest trees are generally difficult to grow and differentiate *in vitro* (BONGA 1982), unless they are obtained from juvenile material. Vegetative propagation of most mature trees is also difficult, unless the cuttings are taken from young trees. Obviously, some change occurs in the physiology/biochemistry of the mature tree that seems to interfere in the rejuvenation

process. European beech (*Fagus sylvatica* L.) is extremely difficult to propagate vegetatively by cuttings from mature trees. The present investigation was aimed at *in vitro* induction of organogenesis in explants derived from juvenile material and mature trees of beech, for eventual application in clonal propagation of superior genotypes.

Materials and Methods

The present study was based on three mature trees (more than 70 years of age) belonging to three cultivars of European beech, *Fagus sylvatica* L., 'Zlatia', 'Pendula', and var. *purpurea*. Bud explants from the mature trees, taken in late autumn and early spring, and embryonal explants derived from seeds collected from the same trees were cultured on a modified Woody Plant Medium (WPM; LLOYD and McCOWN 1981). The WPM was variously supplemented with growth substances: 0.3–1.0 mg/l 6-benzylaminopurine (BA), 0.2–0.5 mg/l kinetin (KIN), 0.01–0.04 mg/l naphthaleneacetic acid (NAA), 0.01–0.02 mg/l indole-3-acetic acid (IAA), 0.01–0.02 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), and 10–20 mg/l adenine sulphate. In addition, 100 mg/l casein hydrolysate and 50–100 mg/l lysine were also tested in combination with phytohormones. Altogether 9 different modifications of WPM involving different combinations of growth substances were employed for the present study. Cultures were maintained at 25°C, 70% relative humidity, and 16 hours photoperiod (2000–3000 lux).

Results and Discussion

Preliminary results from the present study have indicated that bud explants from mature beech trees are, in general, difficult to grow and differentiate *in vitro*. Of the three beech cultivars investigated, bud explants from 'Zlatia' and 'Pendula' showed practically no growth and differentiation on the media tested. However, about 50% of the 206 bud explants from var. *purpurea* showed slight growth, and only a small proportion (about 10%) of the explants exhibited a limited differentiation of buds (mostly one or two) on media containing low levels of phytohor-

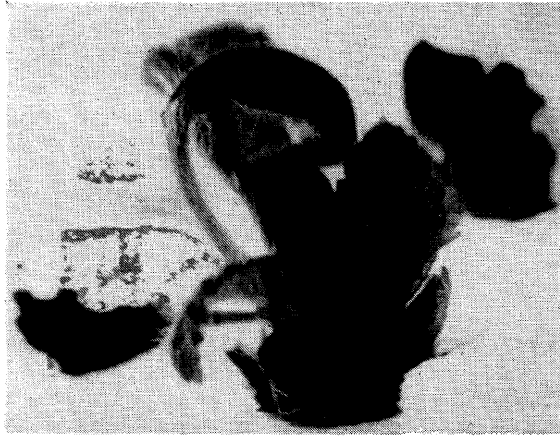


Figure 1. — Differentiation of shoots on a bud explant from a mature (more than 70 years old) *Fagus sylvatica* var. *purpurea* cultured on modified WPM supplemented with 0.5 mg/l BA, 0.02 mg/l NAA, and 0.02 mg/l 2, 4-D.

mones (Fig. 1). The differentiation of shoots on the bud explants in var. *purpurea* occurred in 10 to 12 weeks.

The embryonal (juvenile) explants from all three cultivars of beech showed growth and differentiation on several different combinations of phytohormones tested. Initially, there was some callusing on the embryonal explants. After 4–6 weeks shoots differentiated on the embryonal explants. The number of shoots was mostly limited to one or two per embryonal explant following the first transfer to the fresh medium. However, in one case as many as 6 shoots and in another 9 shoots per explant were observed. In a few cases both shoot and root differentiation occurred on the embryonal explants (Fig. 2). A single plantlet from an embryonal explant is not optimal for clonal propaga-

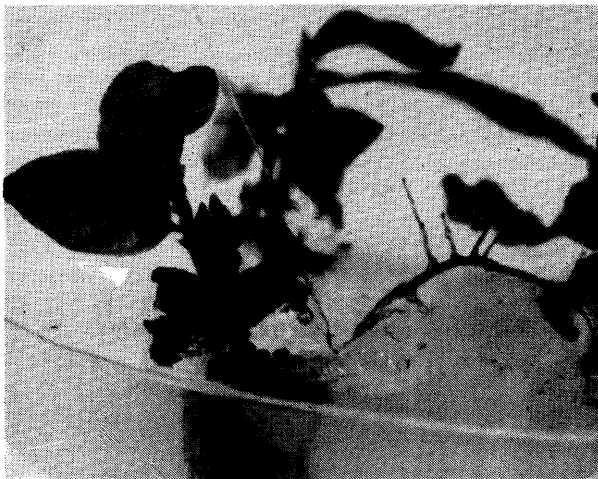


Figure 2. — Plantlet regeneration from an embryonal explant of *Fagus sylvatica* var. *purpurea* cultured on modified WPM supplemented with 0.5 mg/l BA and 0.02 mg/l NAA.

tion. Consistent multiple shoot formation on tissue explants is a prerequisite for cost-effective clonal propagation. We are investigating media and cultural conditions that would allow an increase in the number of shoots per tissue explant.

In these experiments with European beech the embryonal explants from all three cultivars showed growth and differentiation of tissues. However, the number of shoots per explant was rather low, except in sporadic cases. Bud explants from 70 plus years old beech trees from two cultivars, namely, 'Zlatia' and 'Pendula' did not grow or differentiate shoots. However, a small proportion of bud explants from var. *purpurea* differentiated a limited number of shoots under the experimental conditions. These experiments would suggest that juvenile state seems to be morphogenetically more labile as compared to the mature state, which in turn appears to be under differential genetic control.

Tissue culture studies with aspen, *Populus tremula*, *P. tremuloides*, and their hybrids have indicated that rapid clonal propagation can be accomplished by bud meristem explant culture method from mature trees (15 to 40 years old) as well as from juvenile material (AHUJA 1983, 1984). Mature micropropagation has also been accomplished in 100 years old *Sequoia sempervirens* (BOULAY 1979), 100 years old *Tectona grandis* (GUPTA *et al.* 1980), 60 years old *Prunus avium* (CORNU *et al.* 1981), and 10 to 20 years old *Eucalyptus* spp (MASCHEHNAS *et al.* 1982). In this regard, European beech seems to be a recalcitrant tree species. Nevertheless, our tissue culture studies are continuing to probe into problems of multiple shoot production and optimal conditions for root formation in tissues derived from juvenile material and mature beech trees.

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Buchbesprechungen

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Von der dreibändigen Serie „Holzkunde“ ist von dem 1974 erschienenen ersten Band eine überarbeitete 2. Auflage herausgegeben worden. Unter dem Titel dieses Bandes, Mikroskopie und Makroskopie des Holzes, werden der Holzaufbau dargelegt und die

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Das Buch ist in zwei Kapitel gegliedert. Im ersten Kapitel befaßt sich der Autor mit der Biostruktur des Holzes und betont, daß das Wesen dieses Rohstoffes nur aufgrund seiner Entwicklungsgeschichte und der Funktionen seiner verschiedenen Gewebe richtig verstanden werden kann. Die für die Leitung, Festigung und Speicherung gebildeten Gewebe werden ausgezeichnet be-