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In vitro regeneration of Curly birch, *Betula pendula* var. *carelica*

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ABSTRACT: Shoots regeneration and multiplication was achieved by using axillary buds and nodal segments from adult curly-birch as initial explants for the establishment of tissue cultures. Bud explants were grown on WP medium with 1 $mg.l^{-1}$ BAP and 0.05 $mg.l^{-1}$ NAA. Shoot multiplication was very efficient on the mentioned medium with 0.5 $mg.l^{-1}$ BAP and reduced level of inorganic nitrogen. During shoot multiplication adventitious root development was noticed under cytokinin treatment.

KEYWORDS: *Betula pendula* var. *carelica*, BAP, nitrogen, WP medium, BT medium

Introduction

One of the most important applications of "in vitro" culture is micropropagation, which enables also vegetative propagation of adult trees, not usually achieved with traditional methods (SÄRKILAHTI 1989). Rapid asexual multiplication can be achieved by enhanced axillary bud breaking, production of adventitious buds, or somatic embryogenesis (THORPE and PATEL 1984). Hence, nodal segments are particularly appropriate as explants for the clonal propagation of crops, ornamentals and trees, owing to their high multiplication rates, sufficient genetic stability and their requirement for little space per individual (SHARMA and THORPE 1990).

Morphogenesis in organs of higher plants "in vitro" is controlled by the physiological stage of explant donor, mineral nutrition, interaction among exogenous and endogenous phytohormones, and environmental factors including light (TRAN THANH VAN 1981). A major difficulty

in the micropropagation of forest tree species is the poor reactivity of explants taken from adult trees (SÁNCHEZ and VIEITEZ 1991). In spite of it several authors (RYYNÄNEN and RYYNÄNEN 1986, MATSCHKE et al. 1987, SÄRKILAHTI 1988 1989, CHALUPA 1989) have achieved "in vitro" propagation of mature trees of genus *Betula* using axillary and apical buds, nodal segments and shoot tips.

During our experiments we have attempted to regenerate shoots of curly - birch (*Betula pendula* Roth ssp. *pendula* var. *carelica* Merklin) from winter buds and nodal segments. It has been achieved by using a method of shoot multiplication under "in vitro" conditions.

Materials and Methods

The axillary buds and nodal segments from 20 - 22 years old trees were used as initial explants for the establishment of tissue cultures. The material obtained was sterilized with combination of 4 % Chinosol W fungicide solution (60 min.) and 0.1 % solution of mercuric chloride + a few drops of Tween 20 (30 min.). The plant material was subsequently rinsed 3 times with sterile distilled water. The explants were grown on cultivation media - MS (MURASHIGE and SKOOG 1962), MS with modified concentration of macrominerals (SÄRKILAHTI 1988), WPM (LLOYD and McCOWN 1980) and BTM (CHALUPA 1981). Media were supplemented with 6 - benzylaminopurine (BAP) or kinetin in combination with indolebutyric acid (IBA) and naphtaleneacetic acid (NAA). The pH was adjusted to 5.6 - 5.8 with 0.1 N KOH before autoclaving for 20 min. at 121°C. Cultures were grown with 16 h cool white fluorescent lighting (intensity - 5 klx) under temperature of 24°C (day) and 19°C (night). Transferring was carried out each three weeks. For each modification of nutrient media and combination of growth factors 30 buds were used. Experiments were repeated at least twice.

Results

Of the above culture media the best results were obtained by using WPM and BTM with growth factors combination BAP and NAA. MS and modified MS media were not suitable for cultivation of curly - birch explants. First three weeks tissues were grown on media WP or BT without growth factors. After first transferring, buds were cultivated on initiation medium WP (BT) supplemented with auxin and cytokinin for induction of adventitious bud growth. New buds began to develop after 3 - 4 weeks of cultivation on initiation medium. The best results of adventitious bud induction were obtained in a nutrient medium with 1 $mg.l^{-1}$ BAP and 0.05 $mg.l^{-1}$ NAA. Then, vigorous new buds developing explants were transferred to medium supplemented with 0.5

mg.l^{-1} BAP. Later on we used only WP medium because of better results in new shoots growth and adventitious buds formation (Tab. 1). A nitrogen concentration modification was very important during the phase of shoot multiplication. The cultivation of tissues at full concentration of nitrogen was less suitable because new buds and forming shoots grew much slower and separate parts of shoots (basal leaves) became brown and dried up. We modified the nitrogen concentration of NH_4NO_3 and $Ca(NO_3)_2$. In case of the half concentration of organic nitrogen, shoots grew slowly and leaves subsequently died. Full concentration of organic nitrogen and half concentration of inorganic nitrogen improved the growth of new shoots and explants were very vigorous.

After 12 - 14 weeks of cultivation on WPM with 0.5 mg.l^{-1} BAP the first roots began to grow. The number of in vitro rooted explants was 25 % of all cultivated explants.

Discussion

Organ "in vitro" cultures are utilized particularly for fast propagation of plant material and one of the primary goals is the genotype preservation of a tree from which the explant material was taken. In spite of this requirement the largest success was achieved by using juvenile material while information about suitability of genotype of young seedlings is minimal. The youngest tissue from a plant frequently is the best source of explant material (SKIRVIN 1981). Vegetative propagation of adult trees is more difficult that of juvenile ones. Actual

Tab. 1. Differences between adventitious shoot formation on medium WP and BT after 10 weeks cultivation.

Medium	Number of bud/ explants	Number of shoot-producing explants	Number of advent. shoots	Shoots per explant
WPM	240	215	624	2.90
BTM	240	180	380	2.11
MS	240	0	0	0
MS mod.	240	45*	0	0

* - explants died after 5 weeks of cultivation

limitations are not only due to the reduced specific morphogenic capacity but also to the contamination found in field experiments (DÍAZ-SALA et al. 1990).

The curly-grained trait is inheritable, although its genetic background has not yet been determined. It takes about 10 years before it is possible to say whether an individual will become a curly-birch (RYYNÄNEN 1986). On account of described phenomenon it is only needed to use adult trees for micropropagation of curly-birch.

Authors dealing with micropropagation of *Betula* species used several types of nutrient media in the course of explants cultivation. RYYNÄNEN and RYYNÄNEN (1986) during their experiments used several types of media for growth initiation, induction of bud formation and shoot elongation. In our further experiments we have used only one type of nutrition medium for cultivation - WPM. This medium seems to be the most suitable one for the whole process of curly - birch micropropagation.

Unexpectedly, in the phase of shoot multiplication on WP medium with 0.5 $mg.l^{-1}$ BAP, shoots exposed to cytokinin "in vitro" produced adventitious roots. Similar phenomenon have described SHARMA and THORPE (1990). Shoots of *Morus alba* L. maintained on the proliferation medium with BAP as growth regulator for over 4 weeks developed adventitious roots at the basal end. Within the next 10 - 12 days, the roots formed several laterals. TOMAR and GUPTA (1988) observed in vitro organogenesis of *Albizia* species. They pointed out that roots did not develop at any concentrations of BAP or kinetin tried but green compact calli were formed in higher concentrations of cytokinins. We suppose that spontaneous root formation under the cytokinin treatment is possible when the cultivated tissue contains an appropriate level of endogenous auxins. This may be a possible explanation of in vitro shoots rooting on media lacking auxins.

Organ cultures are one of the possibilities which we chose for micropropagation. Further we will try to improve "in vitro" rhizogenesis of separate shoots under an auxin treatment and to transfer complete regenerants to soil after planting the plantlets in greenhouse.

Súhrn

Na založenie pletivových kultúr sme použili explantáty (nodálne segmenty, axilárne púčiky) odobraté z 22 - 25 ročných jedincov brezy svalcovitej. Púčikové explantáty sme kultivovali na niekoľkých typoch kultivačných médií - MS, MS modifikované, VPM a BTM. Médiá boli

doplnené rôznymi koncentráciami auxínov a cytokinínov. Najlepšie výsledky v indukcii adventívnych púčikov, regenerácii výhonkov sa dosiahli na médiu WPM doplnenom BAP v kombinácii s NAA. Rozdielne použité koncentrácie organického a anorganického dusíka v kultivačnom médiu ovplyvnili prežívanie explantátov a rozsah regenerácie. Po 4. až 5. pasáži sme zaznamenali spontánnu tvorbu adventívnych korienkov. V priebehu 3 týždňov sa na vyvíjajúcich korienkoch vytvoril aj jemný vlásočnicový systém.

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