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In vitro propagation of mature trees of *Sorbus aucuparia* L. and field performance of micropropagated trees

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ABSTRACT: The influence of tree age, explant source and genotype on micropropagation of mature trees of *Sorbus aucuparia* has been investigated. Experiments demonstrated the feasibility to use juvenile parts of mature trees for *in vitro* propagation of selected genotypes. Explants from lower branches and from epicormic shoots of mature trees exhibited high multiplication coefficients of microshoots cultured on modified MS agar nutrient medium supplemented with cytokinin (BA, TDZ) and auxin (IBA). Microshoots produced from juvenile parts of mature trees exhibited good rooting response and produced plants were well adapted to grow in forest soils. The survival of micropropagated trees planted in experimental plots was high and losses during winter were low. Height and diameter increments of micropropagated trees originated from juvenile parts of mature trees were considerable and their dimensions after five years of growth were comparable with the dimensions of trees originated from seeds.

Keywords: *in vitro* propagation; *Sorbus aucuparia* L.; mature tree micropropagation; juvenile parts of trees; field growth of micropropagated trees

European mountain ash (*Sorbus aucuparia* L). is a hardy forest tree species, that grows from lowlands to the upper tree line in mountains of Europe. *Sorbus aucuparia* grows well in the harsh climate of high moutain altitudes and thrives on dry as well as moist soils. The height of *Sorbus aucuparia* trees ranges from 10 m to 20 m. Trees yield wood of high technological value that is used for furniture, veneer, pulping, and as firewood. Trees of *Sorbus aucuparia* produce a large quantity of red berries (with a high content of vitamin A and C) which in the variety *edulis* are edible. The fruits are favorite and important food components for birds and game in mountainous regions. They are also used for making jams and alcoholic beverages. *S. aucuparia* tolerates harsh mountainous climatic conditions and is frequently used for reforestation of mountainous regions. Trees of *S. aucuparia* suffer mainly from some diseases caused by fungi and bacteria. The fungus *Phaeolus rutilans* is the injurious fungal disease of *S. aucuparia*. Serious danger to *S. aucuparia* is caused by the bacterium *Erwinia amylovo*ra (Burill) Wins.

Sorbus aucuparia is propagated mainly by seeds, that before germination must be stratified for long period (for 5–7 months in moist sand). Vegetative propagation is an important method for preserving unique characteristics of the selected trees. *S. aucuparia* is a tree species with great variety and occurs in many forms. By the use of *in vitro* technologies it will be possible to achieve rapid propagation of selected elite genotypes and important varieties in a short time. Also the rapid *in vitro* propagation of disease-resistant genotypes and fast propagation of valuable varieties with large edible fruits is desirable.

Our experiments with *in vitro* propagation of *Sorbus aucuparia* showed that *in vitro* technologies can be used for fast micropropagation of important genotypes of *S. aucuparia* (CHALUPA 1981, 1983a,b, 1984, 1985, 1987a,b, 1988, 1990, 1992). This study was carried out to investigate the influence of tree age, explant source and genotype on *in vitro* propagation of mature trees. The effects of plant growth regulators on shoot multiplication were tested and field performance of micropropagated trees was studied.

MATERIALS AND METHODS

PLANT MATERIAL

Nodal segments and shoot tips were used as initial explants for *in vitro* propagation of mature trees. For explant collection, mature trees were selected (45–50 years old) and explants were taken from different parts of mature trees (from branches growing 2 m above ground, or from branches in top parts of trees, or from juvenile parts of trees – from epicormic shoots). For comparison, explants from 10 seedlings (2 years old) and from 10 graftings (branches of mature trees were grafted onto seedlings used as rootstocks), were used. Explants were collected from February to April. Nodal explants were sterilized in

calcium hypochlorite solution (7.5% w/v) for 20 min and then in mercuric chloride solution (0.1% $HgCl_2$) for 20–30 min. After sterilization, nodal segments were washed three times in sterile distilled water and placed on agar nutrient medium.

CULTURE MEDIA AND CONDITIONS

Nodal segments were grown on modified MS medium (MURASHIGE, SKOOG 1962) or on BTM (CHALUPA 1983a). The basal medium was supplemented with glutamine (100 mg/l) and casein hydrolysate (100 mg/l) and 30g/l sucrose. The media were solidified with Difco Bacto agar (7 g/l) and adjusted to pH 5.7, before sterilization by autoclaving 121ºC for 20 min. Growth regulators and glutamine were filter – sterilized. Growth regulators added to nutrient medium included 6-benzylaminopurine (BA), or thidiazuron (TDZ) in combination with indole-3-butyric acid (IBA). BA was tested in concentrations 0.1–4.0 mg/l, thidiazuron in concentrations 0.001–1.0 mg/l. IBA was added in concentration 0.1 or 0.2 mg/l. Microshoots 10–20 mm long were rooted in a modified WPM (half strength) containing low concentration of sucrose (5–10 g/l) and low concentration of auxin (IBA 0.3–0.6 mg/l, NAA 0.2–0.5 mg/l).

RESULTS

EFFECTS OF PLANT GROWTH REGULATORS ON SHOOT MULTIPLICATION

Fast axillary bud proliferation and shoot multiplication of mature trees of *S. aucuparia* was achieved on modified MS medium supplemented with a low concentration of cytokinin (BA or TDZ) plus auxin (IBA). Nodal segments placed on MS agar nutrient medium started to form shoots within 2–3 weeks and many new shoots were produced within 5–6 weeks. Of the nutrient media tested (MS, BTM, WPM) the highest multiplication rate was obtained on modified MS medium. All tested cytokinins (BA, TDZ) stimulated the fast formation and proliferation of new shoots from axillary buds of nodal segments of mature trees (Table 1). MS nutrient medium supplemented with a low concentration of BA (0.2–0.4 mg/l) plus IBA (0.1 mg/l) promoted effectively formation of longer shoots in nodal segments of mature trees. MS nutrient media supplemented with higher concentration of BA (0.6–1.0 mg/l) stimulated formation of numerous shoots, however, the shoots were short.

The shoot formation and proliferation was also stimulated on MS medium containing TDZ as cytokinin, however, the produced shoots were short. Modified MS medium, supplemented with TDZ (0.005–0.01 mg/l) plus IBA (0.1–0.2 mg/l) promoted formation and proliferation of shoots of mature trees of *S. aucuparia*. Experiments showed that shoot proliferation of mature trees of *S. aucuparia* is promoted on MS medium with different cytokinins. In general, the number of shoots produced on media supplemented with cytokinins increased with increasing cytokinin concentration. However, the shoot length decreased with increasing cytokinin concentration. Shoots produced on media containing a higher concentration of cytokinin (BA 1–2 mg/l) or TDZ (0.05–0.2 mg/l) were numerous and short (Table 1).

EFFECTS OF TREE AGE AND EXPLANT SOURCES ON SHOOT PROLIFERATION AND ROOTING

Mature trees are characterized by decreasing capacity for vegetative propagation. However, different parts of mature tree are often in different degree of maturity.

Table 1. Effect of cytokinin on shoot proliferation of mature trees of *Sorbus aucuparia* L.

BA (mg/l)	Number of shoots*	Shoot length (cm)	TDZ (mg/l)	Number of shoots*	Shoot length (cm)
0.2	3.6 ± 1.5	1.8 ± 0.6	0.005	2.7 ± 1.2	1.3 ± 0.4
0.4	4.3 ± 1.8	1.5 ± 0.4	0.01	3.5 ± 1.5	1.1 ± 0.3
0.6	5.9 ± 2.1	1.4 ± 0.4	0.02	4.2 ± 1.9	0.8 ± 0.3
1.0	8.2 ± 2.6	1.1 ± 0.3	0.05	7.8 ± 2.3	0.5 ± 0.2

*Data based on 40 nodal segments (collected from lower branches of mature trees) cultured on modified MS medium supplemented with auxin (IBA 0.1 mg/l) and cytokinin (BA or thidiazuron). Mean ± SD

Table 2. Effect of explant source on shoot proliferation of *Sorbus aucuparia* L.

Explant source	Number of shoots*	Shoot length (cm)
Mature trees (lower branches)	4.2 ± 1.9	1.5 ± 0.5
Mature trees (top branches)	2.7 ± 1.5	1.2 ± 0.4
Mature trees (epicormic shoots)	5.1 ± 2.2	1.7 ± 0.7
Graftings	4.9 ± 2.6	1.7 ± 0.8
Seedlings	6.2 ± 2.4	2.1 ± 0.6

*Data based on 50 nodal segments (collected from 5 trees) cultured on modified MS medium supplemented with cytokinin (BA 0.4 mg/l) and auxin (IBA 0.1/l). Mean ± SD

Table 3. Effect of explant source on rooting of microshoots of *Sorbus aucuparia* L.

Explant source	IBA	IBA + NAA
	rooted microshoots (%)	
Mature trees (lower branches)	62	74
Mature trees (top branches)	52	62
Mature trees (epicormic shoots)	74	82
Graftings	76	78
Seedlings	84	86

*Data based on 50 microshoots rooted on modified WPM supplemented with IBA (0.5 mg/l), or IBA (0.4 mg/l) plus NAA (0.4 mg/l)

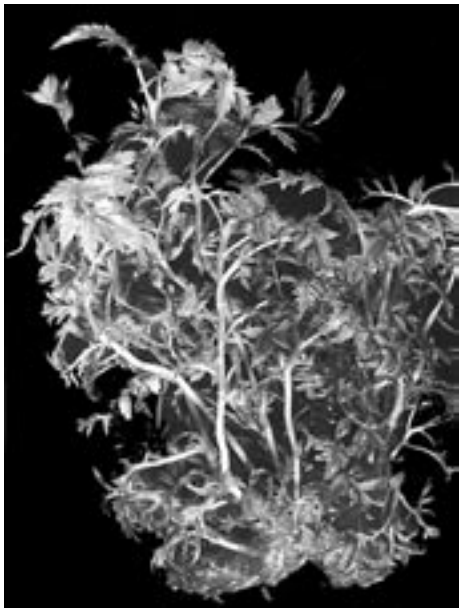

Fig. 1. Shoot multiplication of *Sorbus aucuparia* L. cultured on modified MS medium supplemented with BA (0.4 mg/l) plus IBA (0.1 mg/l)

Fig. 2. Shoot multiplication of *Sorbus aucuparia* cultured on modified MS medium supplemented with BA (0.4 mg/l) plus TDZ (0.01 mg/l) plus IBA (0.1 mg/l)

The basal parts of mature trees often contain tissues with a high degree of juvenility. Vegetative buds produced in young trees may stay dormant for many years. Juvenile tissue may occur in low parts of trees (epicormic shoots and stump sprouts), and new shoots originated from juvenile tissues exhibit juvenile characteristics. *In vitro* propagation of mature trees can be achieved by the use of explants taken from juvenile tissues. Our experiments with explants taken from different parts of mature trees of *S. aucuparia* showed that explant source is an important factor influencing shoot multiplication (Table 2). Explants taken from juvenile parts of mature trees (epicormic shoots, lower branches, graftings), exhibited higher multiplication rates and produced more microshoots than explants from mature parts of trees.

Nodal segments taken from different parts of mature trees and cultured on modified MS medium supplemented with cytokinin (BA or TDZ) plus auxin (IBA) produced new shoots within 4 weeks (Table 2). Shoot cultures were produced from all tested explants, however, multiplication rates of cultures produced from juvenile parts

Table 4. Effect of explant source on height growth of micropropagated trees of *Sorbus aucuparia* L.

Explant source	Height (cm)*	
	2 years	5 years
Mature trees (lower branches)	115 ± 11.4	342 ± 18.1
Mature trees (top branches)	107 ± 12.2	318 ± 19.2
Mature trees (epicormic shoots)	123 ± 11.6	355 ± 17.5
Graftings	119 ± 12.7	352 ± 18.9
Seedlings	128 ± 10.3	368 ± 18.1

*Data based on 15 micropropagated trees. Mean ± SD

Fig. 3. Microshoot of *Sorbus aucuparia* rooted on agar WPM (half – strength) supplemented with IBA (0.4 mg/l) plus NAA (0.2 mg/l)

of mature trees (epicormic shoots, low growing branches) were higher than those from adult parts of mature trees (explants from branches growing in top parts of crown). Multiplication rates of shoot cultures derived from seedlings were higher than those of mature trees (Table 2).

Shoot cultures derived from epicormic shoots and lower branches of mature trees maintained high multiplication coefficient during the whole experimental period without a significant decrease in regeneration capacity. These results indicate that explants from epicormic shoots and lower branches of mature trees can be used for initiation of shoot cultures and for *in vitro* propagation of mature selected trees of *S. aucuparia*.

Microshoots excised from multiplying cultures that were transferred on rooting medium (modified WPM supplemented with auxins) started to form adventitious roots within 2–3 weeks. Microshoots derived from juvenile parts of mature trees exhibited good rooting response (70–80% of microshoots formed adventitious roots in rooting medium within 3–5 weeks – Table 3). Rooted plantlets were transplanted into potting mixture. Some microshoots derived from explants of mature trees were rooted *ex vitro*, in a potting mixture. After auxin treatment, microshoots were inserted into potting mixture (peat and perlite, 1:2 v/v) and were maintained in a warm and humid atmosphere. Within 4–6 weeks, most microshoots rooted. Microshoots rooted in a potting mixture formed roots well adapted for growth in soil.

Rooted plants were grown under high relative humidity and long photoperiod. After 2 weeks, the high relative humidity was reduced to normal values. Rapid stem elongation and formation of new leaves that were anatomically adapted to a low relative humidity, was important for tree survival. After new adapted leaves had formed, the trees were placed outdoors and grown in partial shade.

FIELD PERFORMANCE OF MICROPROPAGATED TREES

Micropropagated trees after rooting and hardening were transferred to selected sites. The planting was usually done in early summer. Planted trees at the end of the first growing season attained a height of 35–58 cm. The survival of micropropagated trees planted in experimental plots was high and losses during the winter were low. Trees withstood winter frosts without significant losses and next spring continued in growth.

During the second growing season, the height increment was considerable and trees attained the height of 100–130 cm by the end of the second growing season (Table 4). Even in the following years, the height and diameter increment of micropropagated trees was high. At the end of the fifth growing season, micropropagated trees, originated from mature trees of *Sorbus aucuparia*, attained a height of 320–350 cm (Table 4). Differences in height of trees originated from different explant sources of mature trees were observed. Explant source influenced the growth of micropropagated trees significantly. The height growth of micropropagated trees originated from juvenile parts of mature trees (epicormic shoots, lower branches of mature trees) was comparable to the growth of trees originated from seeds.

Fig. 4. Micropropagated trees of *Sorbus aucuparia* growing in the experimental plot

DISCUSSION

Recent advances in micropropagation of broadleaved forest tree species (CHALUPA 1979, 1981, 1983a, 1985, 1987b) have opened great opportunities for mass propagation of selected valuable genotypes of forest trees. Promising results have been mostly obtained with micropropagation of juvenile plant material. The capacity of trees to be propagated vegetatively decreases with increasing age and mature trees have usually a low capacity to be propagated vegetatively. The technique used for rejuvenation of mature trees includes severe pruning, grafting of mature scions on juvenile seedling rootstocks, and the use of explant material from juvenile parts of trees (BONGA 1987; FRANCLET et al. 1987; HACKETT 1987; SAN-JOSÉ et al. 1990; VIEITEZ et al. 1994; MEIER, REUTHER 1994; CHALUPA 2000). Selection of explants, composition of nutrient media, concentration of phytohormones, and methods used for micropropagation have significant effects on shoot multiplication rates, rooting and quality of produced trees.

The modified MS medium that we used in experiments stimulated well shoot growth. Macroelements in nutrient medium provided an adequate level of nutrition for fast shoot elongation and multiplication. The best shoot multiplication was obtained on media supplemented with low levels of cytokinin (BA, TDZ) and auxin (IBA). Higher concentrations of cytokinin reduced shoot elongation. Shoot multiplication rates depended greatly on nutrition of explants and evironmental conditions, however, shoot multiplication and elongation was also greatly affected with juvenility of explants. Our recent experiments with different explant sources of *Sorbus aucuparia* indicate that explants taken from juvenile parts of mature trees, exhibit juvenile characteristics. Cultures initiated from these explants showed a high capacity for shoot formation and fast shoot proliferation. Rooting experiments demonstrated the good rooting capacity of microshoots originated from juvenile parts of mature trees. Height and diameter growth of trees produced from explants originated from juvenile parts of mature trees was rapid and after five years of growth in forest experimental plots, dimensions of micropropagated trees of *Sorbus aucuparia* were comparable with dimensions of trees produced from seeds.

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In vitro rozmnožování dospělých stromů *Sorbus aucuparia* L. a růst stromků rozmnožených *in vitro*

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ABSTRAKT: Byl zjišťován vliv stáří mateřských stromů, vliv genotypu a místa odběru explantátů na rozmnožování dospělých stromů *Sorbus aucuparia* L. metodami *in vitro*. Získané výsledky ukázaly, že při použití juvenilních explantátů odebraných z dospělých stromů je možné dosáhnout rychlého množení dospělých stromů metodami *in vitro*. Explantáty odebrané z juvenilních částí dospělých stromů (nízko rostoucí větve, kmenové výmladky rostoucí v dolní části kmene) umožňovaly dosáhnout značného koeficientu množení prýtů na modifikovaném MS médiu obsahujícím nízké koncentrace cytokininu (BA, TDZ) a auxinu (IBA). Prýty vypěstované *in vitro* byly zakořeněny na modifikovaném WPM obsahujícím nízké koncentrace auxinu (IBA, NAA) a po otužení byly zakořeněné stromky vysazeny na venkovní pokusné plochy. Výškový a tloušťkový růst stromků vypěstovaných *in vitro* probíhal podobně jako růst semenáčků; po pětiletém růstu dosáhly stromky vypěstované *in vitro* podobných rozměrů jako stromky vypěstované ze semen.

Klíčová slova: *in vitro* rozmnožování; *Sorbus aucuparia* L.; mikropropagace dospělých stromů; juvenilní části stromů; růst *in vitro* rozmnožených stromků

Jeřáb ptačí (*Sorbus aucuparia* L.) patří k odolným listnatým lesním stromům s rozsáhlým rozšířením v Evropě. Vzhledem k velké odolnosti k drsným klimatickým podmínkám roste od nížin až do vysokých horských poloh. Kromě dřeva poskytuje i velké množství plodů, které jsou významnou součástí potravy ptáků a zvěře v horských oblastech. Vyskytuje se v různých formách, z nichž významné jsou zejména genotypy odolné k chorobám, rychle rostoucí a poskytující velké úrody výživných plodů. Reprodukce jeřábu ptačího metodami *in vitro* má velký význam pro rychlé rozmnožování vybraných hodnotných genotypů. *In vitro* reprodukce je důležitá pro záchranu cenných genotypů a populací.

Prováděné experimenty byly zaměřeny na vypracování vhodných metod reprodukce dospělých stromů *Sorbus aucuparia* za použití *in vitro* metod, které by umožnily rychlé rozmnožení odolných a produktivních genotypů. Byl zejména zjišťován vliv různých fytohormonů na množení prýtů *in vitro* a jejich zakořeňování a růst stromků po jejich vysazení na venkovní plochy. Experimenty byly zaměřeny na zjištění vlivu stáří stromu, místa odběru explantátů a vlivu genotypu na rychlé *in vitro* množení dospělých stromů jeřábu ptačího.

Jako počáteční explantáty pro založení kultur byly použity explantáty z dospělých stromů (40–45 let), které byly odebírány jednak z juvenilních částí dospělých stromů (kmenové výmladky ve spodní části kmene, nízko položené větve), jednak z vrcholových větví. Nodální segmenty dlouhé 1–2 cm byly po sterilizaci vysazeny na agarové živné médium. Kultury byly pěstovány za kontrolovaných podmínek v kultivační místnosti při teplotě 23 °C, při 16hodinové periodě.

Kultury byly pěstovány na MS živném médiu (MURASHIGE, SKOOG 1962). Média byla zpevněna Difco Bacto agarem (7 g/l). Jako zdroj uhlíku byla použita sacharóza (20–30 g/l). Živná média byla sterilizována autoklávováním po dobu 20 minut, tepelně labilní sloučeniny byly sterilizovány filtrací. Z fytohormonů přidaných do živných médií byl testován účinek 6-benzylaminopurinu (BA) a thidiazuronu (TDZ), z auxinů vliv kyseliny indolylmáselné (IBA) a kyseliny 1-naftyloctové (NAA). Zakořeňování prýtů vypěstovaných *in vitro* bylo prováděno jednak v živných médiích zpevněných agarem, která obsahovala exogenní auxin (IBA, NAA), jednak v substrátech, složených ze směsi rašeliny a agroperlitu (1 : 1 v/v). Při zakořeňování prýtů *ex vitro* v substrátech byly báze prýtů ošetřeny pudrovým stimulátorem.

Z testovaných živných médií byl rychlý růst a proliferace prýtů, odebraných z juvenilních částí dospělých stromů, stimulována nejlépe na modifikovaném MS médiu, které obsahovalo nízkou koncentraci cytokininu (BA, TDZ) a auxinu (IBA). Po umístění nodálních segmentů na agarové živné médium se vytvořily z axilárních pupenů nové prýty během 3–5 týdnů. Růst a vývoj kultur byl ovlivňován použitým typem cytokininu a jeho koncentrací v živném médiu. Se stoupající koncentrací BA (0,2–1,0 mg/l) v živném médiu stoupal počet vytvořených prýtů, jejich délka však klesala. MS médium obsahující nízké koncentrace BA (0,2–0,4 mg/l) a IBA (0,1–0,2 mg/l) stimulovalo efektivně vytváření a prodlužování nových prýtů. Rovněž nízké koncentrace TDZ (0,01 mg/l) plus IBA (0,1–0,2 mg/l) stimulovaly vytváření prýtů, které však byly krátké.

Nodální segmenty odebrané z různých částí dospělých stromů vytvářely při pěstování na MS médiu nové prýty, které se dále množily. Množící se kultury se vytvářely u všech zkoumaných explantátů, nejvyšší koeficient množení byl zjištěn u kultur vzniklých z juvenilních částí

dospělých stromů (kmenové výmladky z dolních částí kmene).

Vytváření kořenů u prýtů vypěstovaných *in vitro* bylo stimulováno na agarovém WPM (poloviční koncentrace), obsahujícím nízké koncentrace auxinu (IBA, NAA). Adventivní kořeny se začaly vytvářet na bázi prýtů po přesazení do vhodného živného média. Bylo dosaženo vysokého procenta zakořenění (70–80 %) prýtů vypěstovaných *in vitro*. Zakořenění prýtů vypěstovaných *in vitro* bylo dosaženo i v nesterilním substrátu (směs rašeliny a agroperlitu 1 : 1 v/v) po předchozí stimulaci báze prýtů v pudrovém stimulátoru. Kořeny vytvořené v nesterilním substrátu byly morfologicky i funkčně dobře přizpůsobené pro další růst sazenic v půdě.

Po zakořenění byly stromky vypěstované *in vitro* pěstovány při vysoké vzdušné vlhkosti, která byla postupně snižována na normální hodnoty. Po otužení byly stromky vypěstované *in vitro* vysazeny na venkovní pokusné plochy, kde pokračovaly v růstu, a na konci vegetační doby dosáhly výšky 35–58 cm. Úmrtnost stromků vypěstovaných *in vitro* byla v zimním období nízká, nedošlo k jejich poškození mrazy. Výškový a tloušťkový růst stromků vypěstovaných *in vitro* probíhal podobně jako u stromků vypěstovaných ze semen. Nebyly pozorovány žádné morfologické ani růstové abnormality u *in vitro* množených stromků. Na konci pátého vegetačního období dosáhly stromky vypěstované *in vitro* výšky 320–350 cm. Výška stromků vzniklých z juvenilních částí dospělých stromů (z kmenových výmladků z dolní části kmene) byla srovnatelná s výškou stromků vzniklých ze semen.

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