Clonal Propagation of *Magnolia* × *soulangeana*

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Introduction

Mass propagation of magnolias presents many problems. Various cutting propagation methods have been tried, but without the application of growth regulators, they are ineffective (Bojarczuk 1982, Gardiner 1989). To increase the effectiveness of magnolia propagation, scientists are studying new methods of tissue culture (clonal propagation). When propagating by tissue culture, the success of magnolia regeneration depends on many internal and external factors, such as: the development stage of the initial explant, donor age, culture medium composition and culture conditions (Biederman 1987, Merkle and Wiecko 1990, Kamenicka 1992, Merkle and Walson-Pauley 1993, Callaway 1994, Kamenicka and Valka 1997).

The best known and most widely planted of all magnolias in the Slovak Republic is saucer magnolia [*Magnolia* × *soulangeana* (Soul.-Bod.)]. This hybrid is found in private and botanical gardens, parks and arboreta at elevations of 394 to 2395 ft (120 to 730 m) above sea level (Bencat 1982). April and early May is the normal time for flowering. This paper summarizes our research on clonal propagation of the saucer magnolia.

Materials and Methods

Plant Material, Initiation, and Tissue Culture

The procedures and conditions for the initiation, multiplication, and rooting of shoot culture, starting from juvenile shoots of saucer magnolia were described previously (Kamenicka 1992, Kamenicka and Valka 1997, Kamenicka and Lanakova 2000). For this study, donor plants of saucer magnolia were grown at the Arboretum Mlynany, Slovak Academy of Sciences (Slovak Republic). Initially, defoliated shoots 2–6 inches (6–15 cm) long) were sterilized in 0.1% $HgCl_2$ containing 30 drops per liter of Tween 20 and rinsed three times for three minutes each (for a total of 9–10 minutes) in sterile, de-ionized water.

Multiplication

The apical part of the shoots were removed and aseptically divided into 3 to 4 nodal segments [1–2 inches (3–5 mm)]. They were placed horizontally on basal S medium (Standardi and Catalano 1985) and WPM medium (Lloyd and McCown 1980) supplemented with 2.32 $\mu mol.dm^{-3}$ BA (corresponding to 0.5 $mg.l^{-1}$ BA) (6-benzylamino-purine). After six to eight weeks, the primary culture was transferred to a medium supplemented with 1.33 $\mu mol.dm^{-3}$ and 0.54 $\mu mol.dm^{-3}$ NAA (a-naphtaleneacetic acid), corresponding to 0.3 $mg.l^{-1}$ for BA (6-benzylamino-purine) and 0.1 $mg.l^{-1}$ for NAA-multiplication medium (see Photograph 1). The media was solidified with 0.7% Agar-Agar (Sigma Chemical Comp., St. Louis, USA) and adjusted to pH 5.6 before autoclaving at 250 °F (121 °C) and 108 kPa for 15 minutes. Twenty-five ml of basal medium was poured into 100 ml culture vessels that were covered with plastic light closures (FORING-LOCK BF/ × 82.5 × 24/6). Four to five shoot segments were introduced to each culture vessel. The cultures were maintained at 71.6 °F (22 ± 2 °C) under a 16-hour day, 8-hour night photoperiod of 35–40 $\mu mol.m^{-2}.s^{-1}$ light irradiation and 90% relative humidity.

Rooting

For rooting, individual shoots 0.8–1 inches (2–3 cm) long were harvested at the end of the multiplication stage and transferred to a rooting medium containing half-strength macro-nutrients of basal S medium (see Photograph 2). The rooting medium was supplemented with 2.0

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Photograph 1. After six to eight weeks, the primary culture was transferred to a medium for multiplication.

mg.l^{-1} IBA (4-(3-indolyl) butyric acid), 0.3% activated charcoal, 20 g.dm^{-3} one of the following carbohydrates: arabinose, cellulose, fructose, galactose, glucose, lactose, mannose, rhamnose, ribose, sorbose, sucrose or xylose, and 7 g.dm^{-3} Agar-Agar. All the chemicals were manufactured by Fluka Chemie AG. Fifty milliliters of rooting medium were poured into 370 ml culture jars having translucent caps with vent membranes. Five shoots were placed into each jar. Growth conditions were the same as stated in the previous paragraph.

Results and Discussion

The process most frequently used for commercial micro-propagation is outlined in Table 1. Saucer magnolia cultures, previously initiated and maintained on multiplication medium were transferred to rooting media supplied with carbohydrates. Approximately two weeks after transferring the shoots to the rooting media, chlorosis of the leaves were evident in on those micro-cuttings whose media contained arabinose, rhamnose or sorbose. The leaves of the micro-cuttings growing on these media were small and light green. In contrast, the leaves of the shoots growing in the presence of the other carbohydrates were dark green.

The epidermal cells of leaves developed had strong, watered cells without cuticle and the stomatal guard was unable to regulate the

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Photograph 2. At the end of the multiplication stage individual shoots were harvested and transferred to a rooting medium containing half-strength macro-nutrients of basal S medium.

opening and closing of the stomatal pore. As a consequence, water was rapidly lost after transferring these plants into normal atmosphere. Also, anatomical adjustment after transferring the explants to normal atmospheric conditions went very slowly and was manifested by successive changes of epidermal cells shape and physiological functions. The stomata of the tissue culture plantlets differed from those of the donor; the stomata were open, but did not function. Also, their density was lower compared to the donor plant. Epidermal cells of the tissue culture and plantlets were irregularly shaped and wavy. Epidermal cells leaves of

Table 1 Summary of protocol for plantlet regeneration from nodal segments of saucer magnolia

Stage	**Procedures**
Prepare for micro-propagation	Select appropriate donor plants and excise juvenile shoots
Sterilize shoots	Treat shoots for 3 to 4 minutes with a solution (0.1 % HgCl2) containing 30 drops/liter Tween 20. Shoots are rinsed with sterile de-ionized water (3 times for a total of 9 minutes).
Initiate culture	Cut shoots into 3 or 4 nodal segments 1–2 inches (3-5mm) long and culture on basal S or WPM media having 0.5 mg.l-1 BA.
Multiply shoots	Plant culture explants on multiplication S and WPM media planted 6-8 weeks. The multiplication media is supplemented with 0.3 mg.l-1 BA and 0.1 mg.l-1 NAA.
Induce roots	Induce roots on 0.8–1 inch (2-3 cm) long shoots on half-strength S medium with 2.0 mg.l-1 IBA, 20 g.l-1 carbohydrates (see *Methods and Materials* section) and 0.3% activated charcoal. Maintain shoots for 90 days.
Acclimatize plants	Transfer rooted plants to a well-drained soil (2 parts sand 1 part peat) and grow in a greenhouse at 70–75 ° F (20–24 ° C) and at high humidity.

the donor plant were smooth, isodiametrically shaped and had many trichomes. Similar results were obtained by Elliasson *et al.* (1994).

When shoots were transferred to rooting media using various carbon sources, we observed that arabinose, rhamnose and sorbose were ineffective for rooting saucer magnolia micro-cuttings. On media supplemented with the other carbohydrates as listed in Table 2, roots were produced having different root length: cellulose, fructose, mannose, ribose and xylose produced roots having statistically equal length. However, cellulose and xylose were more effective for root fresh and dry mass (*statistically significant) and shoots produced more branched root systems (see Table 2). Shoots placed on media supplemented with all other carbohydrates were shorter and had lower fresh and dry mass than

Table 2 Effects of carbon sources on rooting of micro-cuttings

Carbohydrate	% Rooted	Total root length (mm)	Fresh mass (mg)	Dry mass (mg)
Arabinose	0	—	—	—
Cellulose	100	100 ± 12.49*	8.2 ± 0.015*	0.92 ± 0.002*
Fructose	100	86 ± 11.27*	3.9 ± 0.013	0.35 ± 0.001
Galactose	94	53 ± 7.71	4.0 ± 0.013	0.42 ± 0.002
Glucose	100	56 ± 7.41	4.1 ± 0.015	0.47 ± 0.002
Lactose	100	67 ± 8.41	4.5 ± 0.007	0.35 ± 0.001
Mannose	93	81 ± 10.43*	4.2 ± 0.018	0.49 ± 0.002
Rhamnose	0	—	—	—
Ribose	95	97 ± 15.27*	2.1 ± 0.011	0.23 ± 0.001
Sorbose	0	—	—	—
Sucrose	87	44 ± 6.44	2.9 ± 0.007	0.22 ± 0.001

the shoots cultured with cellulose and xylose (Kamenicka 1996, 1998). Also, shoots on media containing cellulose and xylose were significantly different in total shoot length in comparison with other carbohydrates.

Roots appeared approximately 13 weeks after transferring the shoots to the rooting media. The percentage of rooted shoots varied from 87% (with sucrose) to 100% (with cellulose, fructose, glucose, lactose, xylose). The negative results obtained from arabinose, rhamnose and sorbose, suggest that these carbohydrates are not efficient for rooting saucer magnolia micro-cuttings. Moncousin (1991) also observed that not all carbon sources stimulate rooting. For saucer magnolia *in vitro* micro-propagation, carbohydrates are very important for producing an effective root system on shoots and for subsequent successful acclimatization to autotrophic conditions.

Rooted plants were transferred to the greenhouse. Air temperatures were maintained at (70–75 °F (21–24 °C) and soil temperatures were from 75–80.5 °F (24–27 °C). Because high atmospheric humidity at this stage is very important for plant survival, polyethylene covers and mist were used to maintain a relative humidity from 80 to 95%.

Ninety percent of rooted plants were successfully acclimatized and established in the greenhouse (see Photograph 3). After an acclimatization period of six to eight weeks, pots containing the plants were transferred to soil and kept at 77–80.5 °F (25–27 °C) and 80–90% relative humidity. Plants were hardened off after two or three weeks, fertilized, and sprayed with fungicides. After six to eight weeks, rooted plants were then transferred to the type of plastic pots used in the commercial production of seedlings. In the spring, these plants were transferred to the hotbed, where the plants were watered, but not weeded. Approximately 85% of these plants survived the transfer to the hotbed and the first growing season. Plant mortality was concentrated in those having extremely wet soil (these plants also exhibited chlorosis of leaves).

The scheme for acclimatizing rooted plants of saucer magnolia is summarized in Table 3.

For the micro-propagation process to be successful, it is necessary to consider differences in individual species and their specific environmental requirements. Between species and hybrids there are differences in formation of shoots. The difference is due to high concentrations of

Table 3 The rooting and acclimatizing of micro-propagated plants

Stage	Procedures
Multiplication	Separate micro-shoots 0.8–1 inches (2–3 cm) long.
Rooting	Add agar, IBA and charcoal to rooting media. Remove rooted micro-shoots from medium. Rinse agar from roots under running tap water and with a fungicide solution.
Acclimatization	Transfer rooted explants to soil substrate or plug to a greenhouse. Keep at a high relative humidity (fog, mist or under a plastic tunnel or polyethylene covers), under controlled conditions (photoperiod, temperature and shade). During acclimatization, leaves from *in vitro* plants fall and new leaves grow, relative humidity is gradually reduced, plants are fertilized. Plants are grown under normal greenhouse conditions. Transfer plants from the soil substrate in the greenhouse to pots in hotbed. Acclimatized plants are: transferred to hotbed, sprayed with fungicides, and watered but not weeded. Plants are grown in full sunlight.

PHOTOGRAPH BY AUTHORS

Photograph 3. Ninety percent of rooted plants were successfully acclimatized and established in the greenhouse

phenolic substances that are loosed at cultivation of the primary explant into the culture media and can inhibit tissue culture growth. Therefore, tissue cultures must be transferred to fresh culture media frequently. Additionally, vitrified shoots, which are very fragile and do not usually root, can form.

The success of regeneration is measured by the quality of plantlets acclimatized to greenhouse and soil conditions. For example, defects in the structure and function of the stomata in plants grown *in vitro* cause rapid dehydration and consequently result in dried leaves. Studying stress mechanisms in *in vitro* culture is very important for the resistance of plantlets to changes in growth conditions too.

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