Direct Regeneration of *Magnolia* spp. Via In Vitro Propagation

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Introduction

Native to many countries, *Magnolia* spp. are valued as ornamental plants and attract the attention of horticulturists and recreational gardeners. Because they are difficult to reproduce by conventional methods (Bärtels 1988, Bojarczuk 1985), alternative reproduction methods are used. According to Merkle and Walson-Pauley (1993), using in vitro methods to regenerate magnolias is promising. Among the advantages of direct regeneration are a high rooting percentage and a continuous production of shoots after years in culture.

In spite of its success, in vitro propagation has problems. The main problem is the transfer of growth inhibiting phenolic compounds from the primary explant to the culture media. These phenolics inhibit growth and are the reason for transferring explants to fresh media every 4 - 6 weeks. The second problem is the tendency of explant vitrification. Two contributing factors to this problem, which cause explants to be translucent and not have roots, are high cytokinin concentrations in the media and high humidity in the culture vessels (Biedermann 1987). The third problem is a decrease in the number of axillary shoots produced after years of continuous growth.

Since 1985, our research has focused on developing regeneration systems for *Magnolia* spp., *Rhododendron* spp.

and *Castanea* spp. and is available in scientific journals and symposia proceedings. This paper summarizes research only on saucer magnolia (*Magnolia* x *soulangiana* Soul. - Bod.).

Results of Magnolia Research

Primary explants were collected in April 1985 from a 100-year-old saucer magnolia plant. Defoliated apical shoots were sterilized for 5 minutes in a solution of 0.1–0.3 % $HgCl_2$ with three drops of Tween 20 (0.03–0.05 %) and cut into 3–5 segments (3–5 mm). The shoots were then placed horizontally on modified Standardi and Catalano (1985) S-medium or modified Lloyd and McCown (1980) WPM-medium in culture vessels. All media were supplemented with 0.01–0.1 mgl^{-1} α-napthaleneacetic acid (NAA), 0.5–0.3 mgl^{-1} 6-benzylaminopurine (BA), 20gl^{-1} sucrose and 7gl^{-1} agar. Tissue cultures were kept in an air conditioned room at 20°C–22°C, 90% humidity and at a light intensity of 35–40 $\mu molm^{-2}s^{-1}$. After 6 weeks, explants were separated into shoots and transferred to fresh multiplication medium or to rooting medium. At each separation, shoots per explant were counted, measured and weighed (Rypak and Kamenicka 1986). Based on this data, the Standardi and Catalano S-full medium supplemented with 0.3 mgl^{-1} BA and 0.1 mgl^{-1} NAA is optimal for shoot multiplication (Kamenicka 1992) (Table 1 and Figure 1). This media should be placed in a culture vessel having a parafilm covering.

Table 1: Effects of different media on the growth and development of saucer magnolia shoots

Media	Mean number of shoots per explant ± SE	Mean length of shoots per explant ± SE (mm)
WPM–full	4.924 ± 0.135	14.936 ± 0.255
WPM–half	4.422 ± 0.134	17.041 ± 0.446
S–full	8.303 ± 0.440	16.239 ± 0.066
S–half	6.167 ± 0.277	16.666 ± 0.335

Figure 1: *Multiplication of saucer magnolia axillary shoots.*

This closure allows light to reach the explants and gas exchange to occur. This combination of medium and vessel covering produces 8.3 axillary shoots per explant at a 90% survival rate (Kamenicka 1996). Media with polyurethane-plastic closures and metal closures produced 5.8 and 4.4 shoots per explant, respectively. Differences in the length of produced shoots were not statistically significant.

After an additional six weeks in various rooting media, shoots and roots were counted, weighed and measured. From this data, the optimal rooting medium is a 50% micronutrient-reduced Standardi and Catalano S-medium containing 4.0 mgl^{-1} indole-3-butyric acid (IBA) and 3.0 mgl^{-1} activated charcoal to stimulate rhizogenesis. Temperature and light intensity were the same as above. Under these conditions,

Table 2: *Characteristics of saucer magnolia stomata*

Type	Size of epidermal cells (mm^2)	Mature stomata per mm^2	Size of Stomata and guard cells	
			length (mm^2)	width (mm^2)
Donor Plant	27	152	17.6	1.5
Tissue Culture	80	70	13	5.9
Regenerant	22	70	17.9	1.4

90% of all shoots survived and 96% of those produced roots.

The regenerants were planted in a 3 sand:1 peat mixture for the next 10–12 weeks kept in a greenhouse at 22°C–24°C and 50% humidity (Figure 2). During the first 10 days in the greenhouse, the leaves fall off and new ones grow. The survival rate in this stage of regeneration is 90%. After this acclimatization, the magnolias were transferred to field conditions. The survival rate at this stage is 87% (Kamenicka and Valova 1994).

Our more recent research has shown that shoots cultivated for one year produce twice as many shoots as those continuously cultivated for 10 years. These shoots also have three times the fresh weight and four times the dry weight as those continuously cultivated for ten years (Kamenicka *et al.* 1996).

We have also found anatomical differences of stomata between the donor plant, the tissue culture and the regenerant (Table 2). The epidermal cells of the tissue culture were 3.6 and 2.9 times larger than those of the regenerant and donor plants, respectively. More notably, the stomata plus guard cell of the tissue culture were 1.3 times shorter, but 4.2 times wider than the donor and regenerant plants. However, the donor plant has 2.1 as many mature stomata per mm^2 as the tissue culture and regenerant (Kamenicka and Lanakova 1996).

Chemical element accumulation of tissue culture leaves,

Figure 2: *Regenerants in the greenhouse.*

stems and callus were different (Kamenicka et al. 1994). In order of the most concentrated to the least concentrated the elements at the beginning of the tissue culture were: potassium, calcium, sodium, magnesium, zinc, manganese, iron, copper. After 20 weeks, the order changed to calcium, potassium, sodium, magnesium, zinc, iron, manganese, copper. That is, calcium (97.9 mgg^{-1}) became more concentrated than potassium (62.7 mgg^{-1}), and iron (1.4 mgg^{-1}) more than manganese (1.2 mgg^{-1}).

Conclusion

Though phenolic compounds, vitrification and aging explants limit the efficiency of the micropropagation system, direct regeneration of *Magnolia* spp. is successful. Based on

our data, one explant produces 5.0 regenerates in 6 months, under optimal growth conditions. Further investigation to increase the survival rate will yield an even more efficient production. Such investigation has begun with examining anatomical differences between the donor plant, tissue culture and regenerant. Our future research will concentrate in these areas.

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