

# Multiple shoot induction and plant regeneration from axillary buds of *Magnolia* ‘Vulcan’

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**Abstract** An efficient protocol for multiple shoot induction and plant regeneration from axillary bud culture of *Magnolia* ‘Vulcan’ was developed in the present study. Primary shoots were obtained from axillary bud explants cultured on Murashige and Skoog (MS) medium containing 1.0 mg/L 6-benzylaminopurine (BA). To induce multiple shoots effectively, primary shoot tips were cultured on MS medium supplemented with different concentrations of BA and zeatin at 0, 0.2, 0.5, and 1.0 mg/L. Of these treatments, the MS medium with 0.5 mg/L BA resulted in the highest number of shoots per explant with an average value of 5.9, and it produced the greatest shoot height at 4.8 cm after 12 weeks of culturing. In the rooting of *in vitro* produced shoots, the greatest percentage of explants forming roots (91.3%), number of roots per explant (9.7), and root length (2.8 cm) were obtained in half-strength MS medium supplemented with 6.0 mg/L indole-3-butyric acid (IBA). Regenerated plantlets were successfully acclimatized and hardened off inside the culture room with 87.5% survival rate. Plants were transferred to a greenhouse with a 97.2% survival rate. The highly efficient shoot multiplication and plant regeneration system reported herein can be used for large-scale clonal propagation of valuable *Magnolia* species or cultivars.

**Keywords** *Magnolia* ‘Vulcan’, tissue culture, shoot multiplication, root induction, BA, IBA

## Introduction

*Magnolia* species are one of the most glamorous ornamental

landscape plants. One of the darkest red magnolias, ‘Vulcan’, is a striking deciduous shrub and elegant tree with rich, ruby-red flowers. ‘Vulcan’ is a hybrid between a *M. liliiflora* hybrid and *M. campbellii* ‘Lanarth’, and is a cultivar gaining popularity in gardens. As demand for ‘Vulcan’ is increasing, the continuous supply of its propagules is necessary.

Magnolias are sexually propagated from seeds and asexually from vegetative tissue. Vegetative propagation through cuttings is used to multiply clonal plants of valuable species and cultivars of *Magnolia*. Mass propagation of magnolias is difficult because the germination rate of seeds is relatively low and cuttings often have poor rooting ability (Callaway 1994; Ming and Huan-Cheng 2003). *In vitro* culture techniques provide an opportunity for large-scale clonal propagation of *Magnolia* species or cultivars that are difficult to produce by conventional methods.

In general, plant regeneration systems through *in vitro* culture using apical or axillary buds are the most applicable method for clonal plant propagation. Bud culture techniques have been successfully applied for the clonal propagation of some species, including *Stevia rebaudiana*, *Citrus reticulata*, and *Enicostema axillare* (Rangappa and Aind 2013; Shende and Manik 2015; Sasidharan and Jayachitra 2017). In *Magnolia* species or cultivars such as *M. sirindhorniae*, *M.* ‘Ann’, *M. obovata*, *M. stellata*, *M.* × *liliiflora* ‘Nigra’, and *M.* × *soulangiana* ‘Coates’, efficient methods for clonal multiplication have been developed using apical or axillary bud cultures (Chaidaroon et al. 2004; Parris et al. 2010; Ana-Maria 2012; Sokolov et al. 2014; Wojtania et al. 2015). However, there have been no reports about clonal propagation using tissue culture methods for *M.* ‘Vulcan’.

The aim of the current study was to optimize a protocol for efficient multiple shoot induction and plant regeneration from axillary buds of *M.* ‘Vulcan’.

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## Materials and Methods

### Plant materials

Axillary buds used as explants for *in vitro* shoot induction were taken from *M. 'Vulcan'* growing at the Chollipo Arboretum in Korea. Stem explants (3 ~ 4 cm long), each containing a node and axillary bud, were sterilized in 70% ethanol for 1 min, disinfected in 3% sodium hypochlorite solution for 20 min, and rinsed five times in sterile distilled water.

### Initiation of shoots from axillary buds

To initiate shoots from axillary buds, nodal segments (approximately 2 cm) containing a single axillary bud were inoculated in MS (Murashige and Skoog, 1962) basal medium supplemented with 1.0 mg/L BA (6-benzyladenine) and 3% sucrose. Media were solidified with 0.3% gelrite powder and adjusted to pH 5.8 before autoclaving for 15 min at 121°C. After inoculation, cultures were incubated at 25 ± 2°C under a 16-h photoperiod with a light intensity of 2,000 lux under white fluorescent tubes.

### Effect of cytokinins (BA or zeatin) on shoot multiplication

The apical portion of the shoot obtained in the initiation phase was used for multiple shoot induction. Excised shoot tip explants were placed on MS medium supplemented with various concentrations (0.2, 0.5, and 1.0 mg/L) of BA or zeatin. Ten explants were established for each treatment. Each treatment was replicated three times. After 8 weeks of culture, the number of shoots per explant was recorded.

### Effect of auxin (IBA) and strength of basal medium salts on *in vitro* rooting

For rooting, shoots approximately 2 cm in length were excised and placed vertically on MS medium supplemented with various concentrations (0.0, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 mg/L) of IBA (Indole-3-butyric acid). To investigate the effect of basal medium salt concentrations on root induction, microshoots were cultured on full-, half-, and one-third-strength MS medium with 6 mg/L IBA. Ten explants were established for each treatment. Each treatment was replicated three times. After 8 weeks of culture, rooting percentage, mean number of roots, and mean root length were recorded.

### Acclimatization

Well-developed plantlets with roots were transplanted to plastic containers (54 × 28 × 6.5 cm) containing artificial soil mixture [perlite, vermiculrite, peatmoss 1:1:1 (v/v)] and were acclimated for 4 weeks at high relative humidity (80–90%). After 4 weeks, survival rates were measured. Twenty plants were planted in soil and each experiment was performed three times. Acclimated plants were in plastic pots and transferred to the greenhouse.

### Statistical analysis

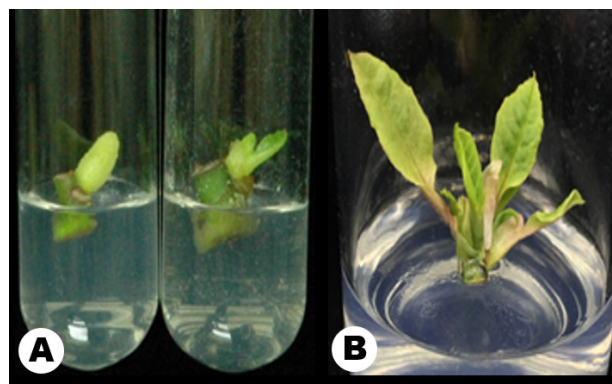
Statistical analysis was performed according to the SAS system (SAS Enterprise Guide 7.1). Means and standard errors were used throughout and the statistical significance of mean values was assessed using ANOVA or Duncan's multiple range tests at  $P < 0.05$ .

## Results and Discussion

### Shoot multiplication

For initiation of primary shoots from axillary buds, explants were inoculated in MS medium supplemented with 1.0 mg/L BA. Explants cultured on the media showed axillary bud emergence and sprouting after 1 ~ 2 weeks of culture (Fig. 1A). Primary shoots were obtained after 6 weeks of culture (Fig. 1B); the shoot tips were then used for further multiplication.

To induce multiple shoots effectively, excised shoot tip explants were cultured on MS medium supplemented with

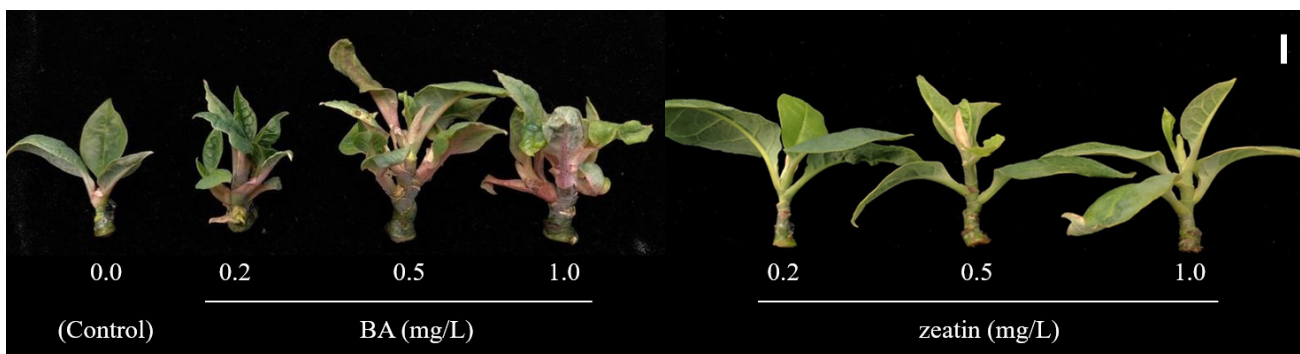


**Fig. 1** The initiation of primary shoots from axillary buds of *Magnolia 'Vulcan'* on Murashige and Skoog (MS) medium supplemented with 1.0 mg/L 6-benzylaminopurine (BA). (A) Axillary bud emergence and sprouting after 2 weeks of culture. (B) Axillary shoot induction after 6 weeks of culturing

**Table 1** Effect of different types and concentrations of cytokinins on multiple shoot induction from shoot tip explants in *Magnolia* ‘Vulcan’ after 8 weeks of culturing

Cytokinin (mg/L)	Average number of shoots per explant	Length of shoots (cm)
Control (growth regulator-free)	1.0 ± 0.0d*	3.4 ± 0.2c
BA 0.2	3.2 ± 0.2c	3.9 ± 0.2bc
BA 0.5	5.9 ± 0.4a	4.8 ± 0.3a
BA 1.0	5.1 ± 0.4b	4.3 ± 0.2ab
zeatin 0.2	1.0 ± 0.0d	4.6 ± 0.3ab
zeatin 0.5	1.0 ± 0.0d	4.9 ± 0.2a
zeatin 1.0	1.0 ± 0.0d	4.8 ± 0.3a

\*Mean separation within columns by Duncan’s multiple range test at 5% level.

**Fig. 2** Multiple shoot formation from apical tips of *Magnolia* ‘Vulcan’ cultured on Murashige and Skoog (MS) medium containing various concentrations of 6-benzylaminopurine (BA) or zeatin after 2 months of culturing. Bar = 1 cm

different concentrations of BA or zeatin (0.0, 0.2, 0.5, and 1.0 mg/L). As shown in Table 1, there were significant differences between BA and zeatin regarding the number of shoots per explant. However, no significant differences were observed in shoot lengths. The highest number of shoots per explant (5.9) was observed in the medium containing 0.5 mg/L BA. The lowest concentration of BA (0.2 mg/L) displayed the lowest number of shoots (3.2). No formation of multiple shoots was seen in the presence of zeatin. Multiple shoots were not induced from shoot tip explants cultured on MS medium supplemented with various concentrations of zeatin. Therefore, BA performed better in multiple shoot induction from shoot tip explants than did zeatin. Thus, BA has a significant beneficial effect on multiple shoot induction of *M. ‘Vulcan’* with an optimal concentration of 0.5 mg/L.

The formation of multiple shoots from shoot tip explants was observed in MS medium supplemented with BA (Fig. 2). The main shoot and axillary buds grew actively and developed into multiple shoots after 8 weeks of culture. Defoliation from the explant was also observed during this period. Unlike BA, the addition of zeatin in the medium

failed to show good shoot multiplication responses as the main shoot grew actively, but axillary buds did not grow into new shoots. During this period, defoliation from the explant was not observed. These results indicate that BA promotes axillary buds to grow a lateral shoot, whereas zeatin promotes shoot elongation, but not shoot multiplication, in *M. ‘Vulcan’*.

Cytokinins have been used to induce multiple shoots from explants, including shoot tip and node explants. The types and concentrations of cytokinin suitable for stimulation of shoot multiplication tend to show different responses depending on the species, including *Stevia rebaudiana*, *Citrus reticulata*, and *Encicostema axillare* (Rangappa and Aind 2013; Shende and Manik 2015; Sasidharan and Jayachitra 2017). Here, there were significant differences in the effect of different cytokinin types (BA or zeatin) and their concentrations on shoot multiplication from shoot tips of *M. ‘Vulcan’*. The greatest multiple shoot induction from shoot tip explants resulted from 0.5 mg/L BA, whereas zeatin at every concentration did not promote shoot multiplication. Similarly, Parris et al. (2010) reported that shoot number was higher on media containing 0.5 mg/L BA,

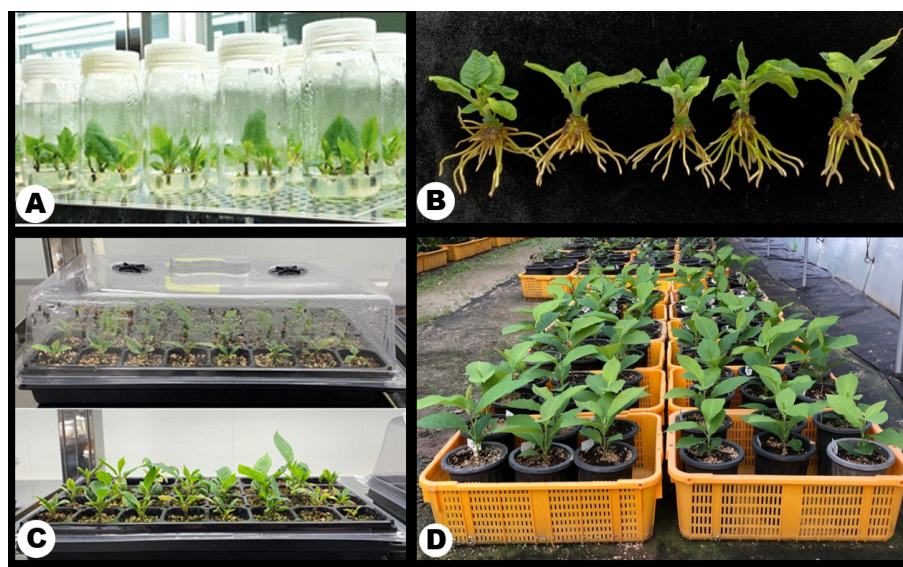
while other cytokinins (mT and 2iP) did not promote shoot proliferation in *M. ‘Ann’*. Radomir (2012) reported that 0.5 mg/L BA was the most effective concentration for shoot multiplication from apical buds of *M. stellate* and *M. × soulangiana* compared to other cytokinins (2iP, TDZ, and kinetin). Wojtania et al. (2015) reported that a concentration of 0.2 mg/L BA resulted in the greatest multiple shoot induction from apical and axillary buds of *M. × soulangiana* ‘Coates’. These results indicate that BA is a crucial growth regulator in shoot multiplication from apical or axillary buds, and its proper concentration varies depending on *Magnolia* species.

### *In vitro* rooting

When *in vitro*-elongated shoots (Fig. 3A) were cultured on

MS medium supplemented with different concentrations (0.0, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 mg/L) of IBA, the appearance of the first primary root was observed after 2–3 weeks of culture. As shown in Table 2, there were significant differences in rooting percentage, number of roots per explant, and root length among the different concentrations of IBA. After 6 weeks of culture, the highest rooting percentages (84.7%) and numbers of roots per explant (8.2) were obtained when the shoots were cultured on MS medium containing 6 mg/L IBA. The greatest root length (2.9 cm) was measured when MS medium was supplemented with 4 mg/L IBA. In MS medium lacking IBA, *in vitro* rooting of shoots was not observed. These results suggest that IBA promotes *in vitro* root formation in *M. ‘Vulcan’* and the optimal concentration is 6.0 mg/L.

To investigate the effect of basal medium salt con-



**Fig. 3** Efficient *in vitro* rooting and *ex vitro* acclimatization of *Magnolia* ‘Vulcan’. (A) Shoots cultured on half-strength Murashige and Skoog (MS) medium containing 6.0 mg/L indole-3-butyric acid (IBA) for root induction. (B) Regenerated young plantlets with well-developed roots after 6 weeks of culturing. (C) Plants acclimatized in plastic containers containing soil for 4 weeks. (D) Cultivation of potted plants in the greenhouse

**Table 2** Effect of different concentrations of indole-3-butyric acid (IBA) on *in vitro* rooting of elongated shoots in *Magnolia* ‘Vulcan’ after 8 weeks of culturing

Concentration of IBA (mg/L)	Rooting (%)	No. of roots per explant	Length of root (cm)
0.0	0.0d*	0.0d	0.0d
1.0	9.5 ± 0.4d	2.6 ± 0.2c	1.9 ± 0.3bc
2.0	12.3 ± 0.6d	4.7 ± 0.6b	2.7 ± 0.4ab
4.0	47.1 ± 0.5b	5.4 ± 0.5b	2.9 ± 0.3a
6.0	84.7 ± 0.4a	8.2 ± 0.6a	2.4 ± 0.2abc
8.0	57.5 ± 0.6b	6.1 ± 0.5b	1.9 ± 0.2bc
10.0	34.6 ± 0.5c	5.1 ± 0.4b	1.5 ± 0.2c

\*Mean separation within columns by Duncan’s multiple range test at 5% level.

**Table 3** Effect of Murashige and Skoog (MS) basal medium salt strength with 6 mg/L indole-3-butyric acid (IBA) on *in vitro* rooting of elongated *Magnolia* ‘Vulcan’ shoots after 8 weeks of culturing

Strength of medium salts	Rooting (%)	No. of roots per explant	Length of root (cm)
Full-strength MS (control)	85.6 ± 0.5a*	8.5 ± 0.4ab	2.4 ± 0.2a
Half-strength MS	91.3 ± 0.4a	9.7 ± 0.5a	2.8 ± 0.2a
One-third-strength MS	88.1 ± 0.6a	7.2 ± 0.4b	1.6 ± 0.1b

\*Mean separation within columns by Duncan’s multiple range test at 5% level.

**Table 4** The hardening and acclimatization of propagated *in vitro* *Magnolia* ‘Vulcan’ plantlets

Stage	Survival (%)	Height (cm)	Length of root (cm)	Fresh weight (g)
Acclimatized plants	87.5 ± 0.5	6.3 ± 0.2	10.3 ± 0.3	4.1 ± 0.2
Potted plants	97.2 ± 0.4	9.7 ± 0.2	24.4 ± 0.5	6.4 ± 0.1

centration on root induction, *in vitro*-elongated shoots were cultured on full-, half-, and one-third-strength MS medium with 6 mg/L IBA. As shown in Table 3, there were no significant differences in percentage of rooting among the different salt strengths. The rooting percentage of shoots cultured on half-strength MS medium with 6 mg/L IBA was slightly higher (91.3%) than shoots cultured on one-third- and full-strength MS medium with 6 mg/L IBA (88.1% and 85.6%, respectively). The greatest number of roots (9.7) and root length (2.8 cm) per explant were observed in half-strength MS medium with 6 mg/L IBA. Consequently, half-strength MS medium with 6 mg/L IBA showed the greatest percentage of explants forming roots, number of roots per explant, and length of roots in *M. ‘Vulcan’*.

Successful rooting of *in vitro*-produced shoots is crucial for facilitating field establishment of micropropagated plants. Supplementation of auxins and the strength of basal medium salts is considered important for rooting of shoots. Auxin plays a critical role in inducing adventitious rooting in many plants. In particular, IBA is widely used for inducing adventitious root formation from *in vitro*-raised shoots of some species, including *Prunus dulcis*, *P. cerasus*, *Dendrocalamus hamiltonii*, and *Pyrus elaeagrifolia* (Tereso et al. 2008; Agnihotri and Nandi 2009; Sarropoulou et al. 2013; Aygun and Dumanoglu 2015). In *Arabidopsis thaliana*, Fattorini et al. (2017) reported that IBA promotes adventitious rooting in thin cell layers by converting IBA into indole-3-acetic acid (IAA). *In vitro* rooting of shoots in some species, such as *Cattleya* and *Passiflora foetida*, is promoted when the overall salt strength of MS medium was reduced (Dewir et al. 2015; Shekhawat et al. 2015). On the other hand, AbdAlla and Mostafa (2015) reported that full-strength MS medium resulted in good rooting of *in vitro*-cultured microshoots of *Rubus fruticosus*. These results indicate that

the effect of MS medium salt strength on rooting of shoots may vary between plant species. In some *Magnolia* species (*M. stellate*, *M. sirindhorniae* Noot. & Chalermglin, and *M. soulangiana*), full- or half-strength MS medium supplemented with IBA alone was used for successful rooting and the optimal concentration of IBA was 4.0 mg/L (Kamenicka and Lanakova 2000; Chaidaroon et al. 2004; Radomir 2012). In the present study, different concentrations of IBA had significant effects on rooting of shoots in *M. ‘Vulcan’*. *In vitro* rooting was the most effective when shoots were cultured in half-strength MS medium supplemented with 6.0 mg/L IBA. At higher concentrations of IBA (up to 10.0 mg/L), the rooting percentage decreased. Thus, the optimal IBA concentration for rooting of *in vitro*-produced shoots may vary between *Magnolia* species ranging from 4.0 to 6.0 mg/L.

#### Acclimatization

The rooted plantlets (Fig. 3B), approximately 2 ~ 4 cm in height, were transferred into plastic containers containing artificial soil for acclimatization. Because high atmospheric humidity is very important for plant survival, the plantlets were covered with plastic caps for approximately 4 weeks to maintain approximately 90% relative humidity (Fig. 3C). The regenerated plantlets were successfully acclimatized and hardened off inside the culture room and showed an 87.5% survival rate (Table 4). The acclimatized plants grew to 6.3 cm in height. Plants were transferred to a greenhouse after 3 months with a 97.2% survival rate and grew to 9.7 cm in height (Table 4, Fig. 3D).

In conclusion, the present study showed that for shoot multiplication, BA was highly efficient at inducing multiple shoots from axillary buds of *M. ‘Vulcan’* and its optimal

concentration was 0.5 mg/L. Furthermore, during plant regeneration, optimal root formation was found when *in vitro*-produced shoots were cultured on half-strength MS medium supplemented with 6.0 mg/L IBA. The highly efficient shoot multiplication and regeneration system for *M. 'Vulcan'* reported here can be used for large-scale propagation of clonal plants.

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