Research paper

Micropropagation through Axillary Bud Culture and Cultivation of *Davidia involucrata* Bail.

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【Summary】

Stem segments with well-developed and dormant axillary buds of 30 *Davidia involucrata* trees were collected as explants for a micropropagation study in January~February 2009 and 2010. These trees, originally from Beichuan Qiang Autonomous County, Sichuan Province, China, were numbered and planted at Fushan Botanical Garden, Taiwan in 2008. Bud dormancy breaking and new shoot production were achieved using axillary buds cultivated on Woody Plant Medium (WPM) supplemented with 0.1~0.5 mg L^{-1} benzyladenine (BA), 1.6 g L^{-1} polyvinylpyrrolidone (PVP), or 100 mg L^{-1} ascorbic acid (AA). For the shoot multiplication, shoot elongation, and rooting study, 5-mo-old *in vitro* shoot tips and nodal segments from trees no. 22 and 28, which produced relatively more buds, were used. Among the different media tested for shoot multiplication, the best result was obtained on WPM basal medium supplemented with 2~3 mg L^{-1} BA. Averages of 5.3~5.6 shoots (shoot tip)$^{-1}$ and 7.6~7.8 shoots (nodal segment)$^{-1}$ were produced. WPM with low concentrations of BA (0.1~0.5 mg L^{-1}) helped shoot elongation. On the best 3 medium compositions (i.e., WPM with 3 mg L^{-1} indole-3-butyric acid (IBA), 1/2 Murashige and Skoog (MS) medium with 2 mg L^{-1} IBA, and WPM with 2 mg L^{-1} IBA and 0.2 mg L^{-1} α-naphthaleneacetic acid (NAA) for *in vitro* rooting, elongated shoots had 100% rooting rates with averages of 9.6~10.6 roots explant^{-1}. Among different individuals, great variations existed in shoot proliferation, elongation, and rooting rates when explants were cultured under the foregoing best medium compositions. Tree nos. 6, 15, 28, and 30 easily produced plantlets by micropropagation, while tree no. 16 had the lowest micropropagation rate. After being acclimatized for 3 wk in a growth chamber (with a survival rate of 95%) and then transferred to a greenhouse at Taipei Botanical Garden for 2~4 mo, some of these 355 surviving plantlets were transplanted to a greenhouses at the Lienhuachih Research Center (63 plantlets) and Meifeng Farm (42 plantlets). After 1 yr, the survival rate at Meifeng was the highest (97.6%), followed by those at Lienhuachih (57.1%) and in Taipei (43.6%). Average heights of plantlets at the 3 sites were similar, at 5.1~5.9 cm. Tissue culture plantlets of tree no. 30 had the highest survival rate and average height among different individuals at all sites.

Key words: *Davidia involucrata*, axillary bud culture, micropropagation, greenhouse cultivation.

Chang SH, Wu CC, Chen FH, Tsay JY, Chen J, Ho CK. 2015. Micropropagation through axillary bud culture and cultivation of *Davidia involucrata* bail. Taiwan J For Sci 30(1):15-29.

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Received August 2014, Accepted February 2015. 2014年8月送審 2015年2月通過。

研究報告

珙桐腋芽微體繁殖與栽植

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摘 要

本研究以2008年12月來自中國四川省北川羌族自治縣，栽植於台灣林試所福山植物園已編號的30株珙桐之形態飽滿的休眠芽作為試驗材料。分別在2009與2010年的1至2月間採集腋芽，培養於添加0.1~0.5 mg L^{-1} benzyladenine (BA)、1.6 g L^{-1} polyvinylpyrrolidone (PVP)或100 mg L^{-1} ascorbic acid (AA)的Woody Plant Medium (WPM)培養基，均可打破芽體休眠，萌發新莖芽。取無菌芽體較多之22與28號苗木培養5個月之試管莖芽的頂芽與節莖進行芽體增殖、抽長與發根試驗，結果芽體增殖以WPM添加2~3 mg L^{-1} BA的處理最佳，節莖增殖率高於頂芽。平均每個頂芽與節莖分別可產生5.3~5.6個與7.6~7.8個多芽體，將多芽體培養於WPM添加低濃度0.1~0.5 mg L^{-1} BA培養基，芽體抽長後，切下2公分莖芽培養誘導發根，最佳的3個發根培養基為WPM添加3 mg L^{-1} indole-3-butyric acid. (IBA)、0.2 mg L^{-1} α-naphthaleneacetic acid (NAA)，與1/2 Murashige and Skoog (MS)培養基添加2 mg L^{-1} IBA，其莖芽都可100%發根，平均每個莖芽的根數為9.6~10.6。將此試驗之最佳培養基，用於其餘編號珙桐芽體之微體繁殖。結果不同單株間的芽體增殖、抽長與發根能力有差異頗大，較易以微體繁殖方法來大量育苗的單株為6、15、28、30號，最困難的是16號。組培苗出栽生長室馴化3週的成活率可達95%以上。台北溫室培育2至4個月之355株小苗，其中63株移到蓮華池，42株與梅峰農場溫室培養，一年後梅峰農場的小苗成活率最高為97.6%，蓮華池次之57.1%，台北最低43.6%。而三地小苗之平均苗高在5.1~5.9公分間。不同單株組培苗生長差異大，以編號30號在3個地區的成活率與苗木高度表現最佳。

關鍵詞：珙桐、腋芽培養、微體繁殖、溫室栽植。

張淑華、吳家禎、陳芬蕙、蔡錦瑩、陳媜、何政坤。2015。珙桐腋芽微體繁殖與栽植。台灣林業科學30(1):15-29。

INTRODUCTION

Davidia involucrata Bail. of the Davidiaceae, also called dove tree or handkerchief tree, is a moderate deciduous tree (with a height of up to 25 m). Its native habitat is in foggy mountains at 700~2500 m in elevation with an annual average temperature of 8~12.4℃. It grows better in semi-shaded and neutral to acidic humus soil rather than barren, arid, and full-sun areas. It grows slowly in the seedling stage (Zhang et al. 1995, 2000, Yu et al. 2006). *Davidia involucrata*, as a relic species of the Tertiary period, was once distributed all over the world; however, now it is endangered and listed under class 1 in the *China Plant Red Data Book* (Fu and Jin 1992). Currently, it only grows in moist subtropical areas of the Yangtze River basin, such as Sichuan, Yunnan, Guizhou, Hubei, Hunan, Shaanxi, and Gansu Provinces of China (27°1'~31°7'N, 98°6'~111°1'E) (He et al. 1995, Chen and Su 2011). A 2010 survey showed that this species was facing severe population declines and was almost extinct in some areas (Chen and Su 2011).

Davidia involucrata has become endangered due to multiple natural and human-

related causes. Besides of its poor natural regeneration, low survival rate after introduction, and weak adaptation to environmental change, *D. involucrata* also has serious reproductive difficulties, including a long juvenile period (15~20 yr), low seed-setting rate but high seed-barren rate, a long dormancy-breaking period (2~3 yr), and a low germination rate (3%) (Zhang et al. 2003, Tang et al. 2004, Su and Su 2005). Therefore, cutting is the major method of asexual reproduction, although rooting success is very low (5%), and the rooted cuttings die if transplanted too soon (Chen et al. 1998).

Tissue culture is an alternative way of asexual reproduction. There were a few successful cases with *D. involucrata* (Wang and Shen 2010). However, most of them were at the initial stages; for example, induction of calli (Luo 2006, Dong and Li 2007, Li et al. 2007b, Mao et al. 2010) and embryo germination and propagation (Dong et al. 2004, Li et al. 2007a, Yu et al. 2009). Only a very few studies focused on using buds to reproduce *D. involucrata*. Zhang et al. (2007) reported that they successfully induced bud-break and callus formation from mature buds, but failed to develop complete plantlets. Although buds from young trees can be used for reproduction, the results greatly varied among individuals (Sato 1996, Jin et al. 2007, Chang et al. 2011).

Thirty 2~3-yr-old *D. involucrata* bare-rooted trees, originally from Beichuan Qiang Autonomous County, Sichuan Province, China, were sent to Taiwan along with 2 pandas in December 2008. After quarantine examination, these trees were numbered and planted in pots at the Fushan Research Center, Taiwan Forestry Research Institute (TFRI) the next day after arriving in Taiwan. These trees had an average height of 3.4 m, diameter at breast height (DBH) of 2.2 cm, and well-developed and dormant axillary buds. One year later, tree no. 15 was dead.

It is important to observe the survivorship and growth of these *D. involucrata* in Taiwan, where all of the environmental factors greatly differ from those of their original habitat. Furthermore, in case these *D. involucrata* die due to an inability to adapt to a sudden change of environment, developing suitable methods to replicate these trees for conservation is essential. In the present study, we conducted research on *D. involucrata* micropropagation through axillary bud culture and a preliminary adaptation test after plantlets were transplanted to greenhouses at Taipei Botanic Garden, the Lienhuachih Research Center, Taiwan Forestry Research Institute (TFRI), and Meifeng Farm.

MATERIALS AND METHODS

Plant materials

Twigs with well-developed and dormant buds were collected from 30 *D. involucrata* plants as explants in January~February 2009 and 2010 (Fig. 1). The twigs were immediately packed in wet paper, stored in a container with ice, and transported to a laboratory in Taipei, and then were kept in a 4℃ refrigerator for less than 1 wk before being cultured *in vitro*.

Axillary bud cultures

Twigs were cut into 10-cm lengths, gently cleaned with a soft brush and liquid detergent, and washed under running tap water for 30 min. Axillary buds were excised, then surface-disinfected with 70% (v/v) ethanol for 1 min, followed by 1% (v/v) sodium hypochlorite in an ultrasonic bath for 15 min. Under laminar flow, sterilized buds were rinsed with sterile distilled water 4 times. After removing the outermost bud scales, the buds were

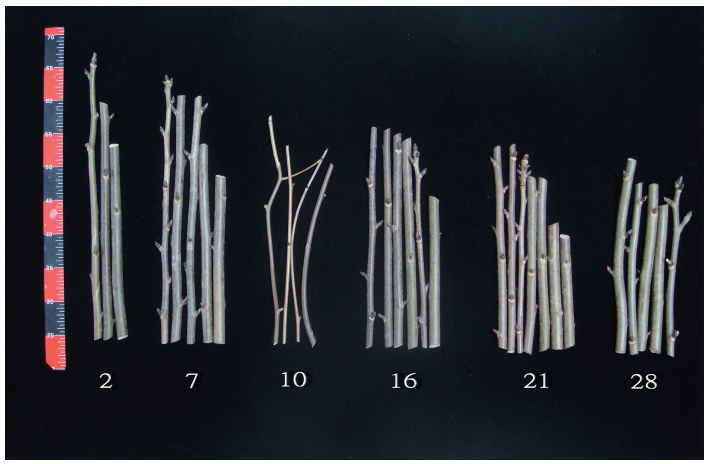

Fig. 1. Twigs with axillary buds from 6 individual trees of *Davidia involucrata* collected on 9 January 2009 and used as explant sources.

cut into 0.8-cm lengths, then cultured on Murashige and Skoog (1962) medium (MS) and Woody Plant Medium (WPM; Lloyd and McCown 1981) supplemented with 30 g L^{-1} sucrose, 0.1~0.5 mg L^{-1} benzyl adenine (BA), 0~1.6 g L^{-1} polyvinylpyrrolidone 4000 (PVP), 0~1.5 g L^{-1} activated charcoal (AC), and 0~0.1 g L^{-1} ascorbic acid (AA). Percentages of survival and contamination were surveyed after 40 d of culture.

Established bud cultures of all 30 plants were subcultured every month on WPM with 30 g L^{-1} sucrose, 0.1 mg L^{-1} BA, and 1.6 g L^{-1} PVP (called WA medium).

Micropropagation of tree nos. 22 and 28

After 5 mo of cultivation, shoot tip and nodal segments from tree nos. 22 and 28, which had relatively more well-developed buds, were collected for a micropropagation experiment. Three buds from each tree (nos. 22 and 28) were cultured on WPM or BW medium (Sato 1996) with 30 g L^{-1} sucrose, and the addition of various plant growth regulators (0~3 mg L^{-1} BA and 0~0.1 mg L^{-1} α-naphthaleneacetic acid (NAA). Each treatment was replicated 4 times. In 6 wk of culture, explants producing multiple shoots and shoot numbers were recorded.

Buds from multiple shoots were then cut and cultured on WPM, 1/2 BW, and 1/2 MS medium with 30 g L^{-1} sucrose, 0.1 mg L^{-1} BA, and 0~1.5 g L^{-1} AC to facilitate shoot elongation. To understand the effect of the basal medium for rooting, 5 elongated buds (15~20 mm long) from each tree (nos. 22 and 28) were used in each treatment, including WPM, BW, 1/2 BW, MS, or 1/2 MS medium with 30 g L^{-1} sucrose, 0~20 mg L^{-1} auxins (NAA or indole-3-butyric acid (IBA)), and 0 or 1.5 g L^{-1} AC. Each treatment was replicated 3 times. Shoot elongation (shoot >1.5 cm) and rooting percentages were recorded after 25 d.

Comparison of the micropropagation abilities of different individual trees

To compare the micropropagation abilities of the 30 *D. involucrata* trees, the same methods as described above were employed. Buds were cultured on WA medium for shoot proliferation. For shoot elongation, WPM with 30 g L^{-1} sucrose and 0.5 g L^{-1} BA was used. WPM with 30 g L^{-1} sucrose and 3 mg L^{-1} IBA was used for the rooting test.

Acclimatization and transplantation

For acclimatization, plantlets were removed from culture tubes when they had grown to 3~4 cm in height. After being washed with water to remove agar from the roots, plantlets were transferred to plug trays filled with perlite and vermiculite (1:1). To retain moisture, plug trays were covered with a transparent plastic cloth for 3 wk in a growth chamber, and moved to a greenhouse at Taipei Botanic Garden, TFRI, with a fog irrigation system (fogged 15 s every 10 min) for 1 mo. The acclimatization processes were repeated monthly in 2011.

All acclimated planted were then moved to another greenhouse. The irrigation frequency was 1~3 times d^{-1} according to the season and weather. In May 2011, some of the plantlets (2~4 mo old) were transferred from the greenhouse in Taipei to greenhouse at Lienhuachih (63 plantlets) and Meifeng Farm (42 plantlets). The remaining 250 plantlets continued growing in the Taipei greenhouse. The growth and survival rate at the 3 locations were compared after 1 yr. Environmental and elevation information of the 3 locations shown in Table 6.

Medium preparation, culture conditions, and data analysis

All media were adjusted to pH 5.7±0.05 using 1 N KOH or HCl, and 7.5 g L^{-1} agar (Difco agar) was added. Flat-bottomed test tubes at 12 cm high with 15 ml of medium used for culture were autoclaved for 15 min at 121℃ and 1.05 kg cm^{-2}. Micropropagation and acclimation were maintained in a growth chamber at 25±2℃ and a 16-h photoperiod provided by fluorescent lights (45 μE m^{-2} s^{-1}).

Data were analyzed with general linear models (GLMs) in SAS software (SAS Institute 1995). To analyze the rooting percentage, data was transformed using angular transformation before carrying out the GLMs.

RESULTS AND DISCUSSION

Culture establishment and shoot sprouting

Our preliminary experiment showed that the contamination and browning rates of unsprouted buds were lower than those of sprouting ones, and survival rates were 92.2% for unsprouted buds and 56.8% for sprouting buds (Chang et al. 2011). Accordingly, unsprouted, dormant but well-developed buds

Table 1. Effects of medium composition on bud sprouting, browning, and defoliation of *Davidia involucrata* axillary bud culture for 40 d

Basal medium	Medium composition				Total no. of explants	Bud sprouting (%)	Browning (%)	Defoliation (%)
	BA mg L^{-1}	PVP g L^{-1}	AC g L^{-1}	AA mg L^{-1}				
WPM					49	0.0	-	-
WPM	0.1				49	100.0	24.5	6.1
WPM	0.1	1.6			47	100.0	4.3	0.0
WPM	0.1		1.5		49	20.4	10.0	10.0
WPM	0.1			100	50	100.0	4.0	0.0
WPM	0.5	1.6			49	100.0	4.1	0.0
MS	0.1				50	96.0	22.9	75.0
MS	0.1	1.6			48	93.8	4.4	88.9

AA, acetic acid; AC, activate charcoal; BA, benzyladenine.

were used in the present study. Results of the effect of the medium composition on axillary bud cultivation are shown in Table 1. Leaves unfolded after 7~10 d of cultivation, while buds began sprouting after around 20 d (Fig. 2a). The contamination rate was < 2%.

BA is a crucial factor in the release of bud dormancy. Sprouting rates on medium with 0.1~0.5 mg L^{-1} BA were >93%, except those on medium containing AC. AC might have absorbed the BA, leading to a lower sprouting rate. On medium without BA, leaves began to unfold, but buds stopped developing or even died afterwards. Zhang et al. (2007) also considered new leaves and growth of apical buds, instead of leaves unfolding of propagated buds, as true indicators of *D. involucrata* shoot sprouting.

Although sprouting was observed on medium with BA, 24.5% of those buds died due to browning, which is a common problem when propagating woody plant buds. Supplementation with PVP, AC, and AA reduced the occurrence of bud browning. Among all medium compositions in this research, medium containing 1.6 mg L^{-1} PVP and 100 mg L^{-1} AA had the lowest bud browning rate of 4%, followed by medium containing AC (with a 10% bud-browning rate). In studies of bud propagation of *Cinnamomum kanehirai* (Chang et al. 2002) and *Musa sapientum* (Ko et al. 2009), PVP and AA were also reported to be able to efficiently reduce bud-browning problems. However, in the case of *D. involucrata*, bud browning only occurred in the early stage of cultivation. Once new buds had

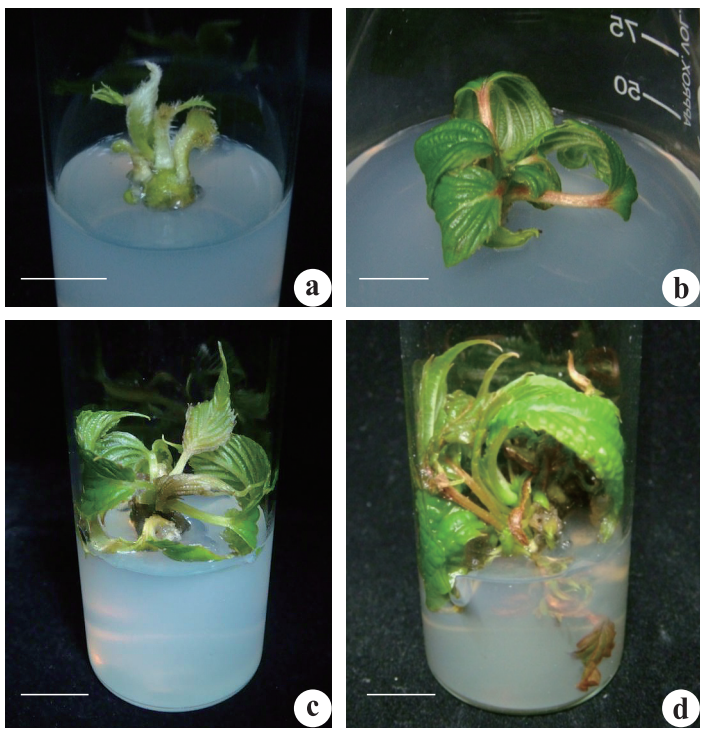

Fig. 2. Bud culture and shoot multiplication of *Davidia involucrata*. (a) Bud sprouting from an axillary bud; (b, c) shoot cultured in WPM (b) vs. MS (c) medium (+ 0.1 mg L^{-1} BA + 1.6 g L^{-1} PVP; (d) shoot multiplication.

sprouted, the browning problem seldom occurred and plantlets usually multiplied and grew healthily.

The bud-sprouting and bud-browning rates of explants cultured on MS medium and WPM were similar. New leaves developing from sprouted buds cultivated on WPM were normal with a dark reddish-green color (Fig. 2b), but those cultured on MS medium were light-green and vitrified, and had callus formation or serious defoliation problems (Fig. 2c). This shows that MS medium with high salinity was not suitable for *D. involucrata* bud cultivation. Yu et al. (2009) also reported that *D. involucrata* embryos developed normally on 1/2 MS medium, but cotyledons did not become green after unfolding on MS medium.

Micropropagation from *in vitro* shoot tips and nodal segments: tree nos. 22 and 28 Shoot multiplication

After 6 wk of cultivation, shoot tips and nodal segments of tree nos. 22 and 28 had produced multiple shoots only on media with plant growth regulators (PGRs) (Table 2). Among all treatments, shoot multiplication rates of either axillary buds or nodal segments cultured on medium with 2~3 mg L^{-1} BA added were the highest (at >90%). Nodal segments are a better material for shoot multiplication than shoot tips. Average shoot numbers per explant were 5.8 and 8.4 for shoot tips and nodal segments, respectively (Fig. 2d), while 1 explant might produce as many as 15 shoots.

For buds from shoot tips, an explant could produce up to 5.4~5.8 multiple shoots, and the effects of different basal media were insignificant when 2~3 mg L^{-1} BA was added. In contrast, nodal segment explants cultured on WPM had higher shoot numbers (8.4 shoots explant^{-1}) than on BW medium (6.8 shoots explant^{-1}). The concentration of BA is one of the main factors for inducing multiple shoots. In our study, both the number

Table 2. Effects of basal medium and plant growth regulators on shoot multiplication of shoot tip and nodal segment cultures of tree nos. 22 and 28 of *Davidia involucrata* after culturing for 6 wk

Basal medium	Plant growth regulators (mg/L)		Percent producing multiple shoots (%)		Mean shoot number per explant	
	NAA	BA	Shoot tip	Nodal segment	Shoot tip	Nodal segment
WPM	-	-	0	0	0.5^{d}	0^{e}
WPM	-	0.5	40	50	2.1^{c}	1.8^{d}
WPM	-	1.0	75	90	3.8^{bc}	4.2^{c}
WPM	-	2.0	100	100	5.3^{a}	8.2^{a}
WPM	-	3.0	95	100	5.4^{a}	8.4^{a}
WPM	0.1	0.5	20	60	2.2^{c}	2.1^{d}
WPM	0.1	1.0	20	75	2.3^{c}	2.2^{d}
WPM	0.1	2.0	95	95	5.8^{a}	7.8^{a}
WPM	0.1	3.0	90	100	5.6^{a}	7.6^{a}
BW	-	1.0	55	70	1.9^{c}	2.1^{d}
BW	-	2.0	95	90	4.8^{b}	5.6^{b}
BW	-	3.0	100	100	5.5^{a}	6.8^{ab}

Means in a column followed by the same letter do not significantly differ ($p < 0.05$) according to Duncan's test.

of multiple shoots and percentage producing multiple shoots were higher when explants were cultured on medium with lower concentrations of BA (0.5~1 mg L^{-1}). Adding NAA (0.1 mg L^{-1}) to the medium did not improve multiple-shoot production. Jin et al. (2007) also suggested that supplementing BA increased multiple-shoot production by 3.4 shoots $explant^{-1}$.

Shoot elongation and rooting

Elongation of *D. involucrata* multiple-shoot clusters is generally problematic. Sato (1996) used 7 subcultures to obtain elongating shoots for further rooting studies. In this research, WPM with BA (0.1~0.5 mg L^{-1}) was effective for shoot elongation, while no shoot elongation was observed on other medium combinations (Fig. 3a).

Elongated buds (15~20 mm long) began rooting after 10 days of culture. Although there were great variations in rooting performances among different medium combinations, *D. involucrata* buds could generally be rooted on media containing IBA (Table 3, Fig 3b). On the 3 medium compositions (i.e., WPM with 3 mg L^{-1} IBA, 1/2 MS medium with 2 mg L^{-1} IBA, and WPM with 2 mg L^{-1}

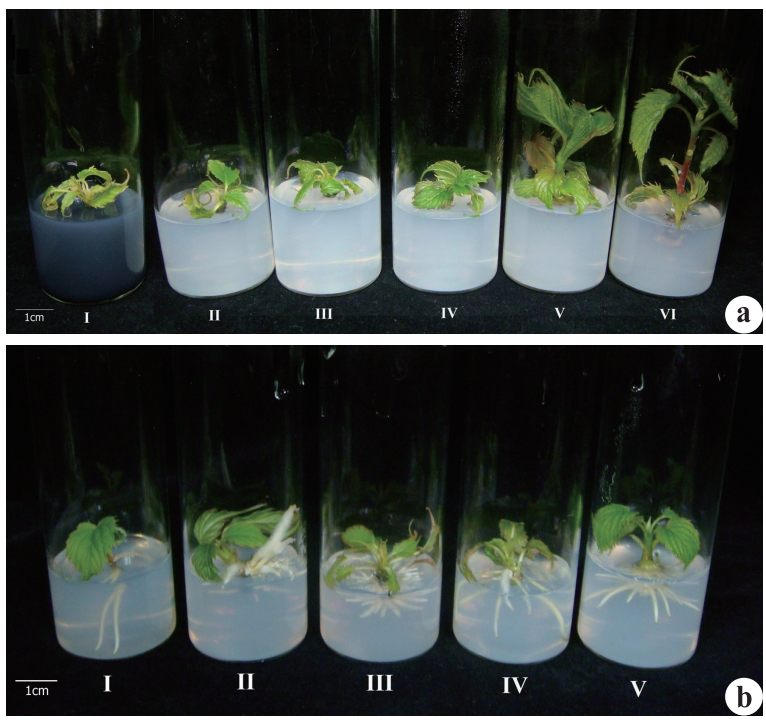

Fig. 3. Shoot elongation and rooting of *Davidia involucrata*. (a) Shoot elongation on different medium compositions: tube I contained WPM with 0.5 mg L^{-1} BA and 1.5 g L^{-1} AC; tube II contained 1/2 MS medium with 0.5 mg L^{-1} BA; tube III contained 1/2 BW medium with 0.5 mg L^{-1} BA; and tubes IV to VI contained WPM medium with 1.0, 0.5, and 0.1 mg L^{-1} BA, respectively. (b) Shoot rooted in rooting media with different compositions: tube I contained WPM with 0.5 mg L^{-1} IBA; tube II contained BW medium with 1 mg L^{-1} IBA; tube III contained 1/2 MS medium with 2 mg L^{-1} IBA; tube IV contained WPM with 3 mg L^{-1} IBA; and tube V contained WPM with 2 mg L^{-1} IBA and 0.2 mg L^{-1} NAA).

IBA and 0.2 mg L^{-1} NAA) with the best rooting performances, healthy shoots had few calluses, 100% rooting rates, and 9.6~10.6 roots $explant^{-1}$. Some other medium compositions were also suitable for rooting, including BW medium with 1~2 mg L^{-1} IBA (90% rooting rate and 10.2~11.1 roots $explant^{-1}$) and 1/2 BW medium with 2 mg L^{-1} IBA (100% rooting rate and 8.1 roots $explant^{-1}$). Medium containing only NAA is not suggested since shoots produced many calluses and had low rooting rates.

WPM and 1/2 MS medium were better basal media than MS, 1/2 BW, or BW media for rooting, which indicates that *D. involucrata* prefers low-salt media. The same results were reported by Jin et al. (2007) and Sato (1996). Adding 1.5 g L^{-1} AC was unfavorable, as plantlets were less healthy, and both the rooting rate and root number declined. This result differed from those of previous reports (Sato 1996, Jin et al. 2007). Various *D. involucrata* tree materials and basal media used may have resulted in these differences.

Micropropagation of different individuals

There were great differences in shoot proliferation, elongation, and rooting abilities

Table 3. Effects of basal medium, auxins, and activated charcoal (AC) on the rooting percentage and root number of *in vitro* shoots of *Davidia involucrata* after 25 d of culture

Basal medium	Auxin (mg/L)		AC	Rooting	Mean root number	Callus size
	IBA	NAA	(g/L)	(%)	per explant	
WPM	0	0		0[e1)]	0[f]	-[2)]
WPM	0.5			80[b]	3.5[d]	-
WPM	1.0			80[b]	10.8[a]	+
WPM	2.0			100[a]	7.8[b]	+
WPM	3.0			100[a]	9.6[a]	+
WPM	4.0			100[a]	5.5[c]	++
WPM	5.0			65[c]	3.3[d]	+++
WPM	2.0		1.5	20[d]	1.0[e]	+
WPM	20.0		1.5	80[b]	1.0[e]	+
WPM	2.0	0.2		100[a]	10.4[a]	- ~ +
WPM		2.0		10[d]	1.2[e]	+++
WPM		3.0		0[e]	0[f]	++++
1/2 MS	2.0			100[a]	10.6[a]	- ~ +
MS	2.0			60[c]	5.3[c]	+
1/2 BW	1.0			80[b]	8.7[ab]	- ~ +
1/2 BW	2.0			100[a]	8.1[ab]	++
1/2 BW	2.0	0.2		100[a]	2.8[d]	++
BW	1.0			90[ab]	11.1[a]	+
BW	2.0			90[ab]	10.2[a]	+++
BW	2.0	0.2		90[ab]	2.6[d]	++
BW	20.0		1.5	70[c]	2.9[d]	-

[1)] Means in a column followed by the same letter do not significantly differ ($p < 0.05$) according to Duncan's test.

[2)] -, +, ++, +++ respectively indicate no, small, medium, and large calli.

among the 30 individuals (Table 4). Tree nos. 6, 15, and 28 had the highest shoot proliferation rates (6.1~6.6 shoots explant^{-1} on average), while tree nos. 8 and 14 had the lowest ones (1.1 shoots explant^{-1}). Nine *D. involucrata* individuals had 100% elongation rates. Tree no. 16, with very a low elongation rate (3.3%) and micropropagation rate, was the only individual which failed to establish rooted tissue culture propagation.

The majority of individuals had >75% rooting percentages, except tree no. 13 (10%), and tree nos. 8 and 9 (35~45%). Since tree nos. 13, 8, and 9 had low shoot proliferation and elongation rates, they were very difficult to regenerate by micropropagation apart from tree no. 16. On the other hand, tree nos. 6, 15, 28, and 30 were easy to regenerate by micropropagation, and over 300 plantlets of these individuals were produced. Jin et al. (2007) also reported that the rooting rate of *D. involucrata* bud multiplication was about 0~89.3% and was affected by medium compositions and individuals.

Acclimatization and culture in the greenhouse

Except for tree no. 16, all rooted plantlets in culture tubes were transplanted to plug trays in a growth chamber (Fig. 4a). After 3 wk, the survival rate was >95% (Fig. 4b). The best months for transplanting to a greenhouse were March and April, as the survival rates after 1 mo were 95~98%. The most improper transplantation time was in June to August, when survival rates were only 40~50%. Survival rates of other months were 60~80% (Table 5). In summer, strong sunlight usually damaged the leaves of plantlets. Although the survival rate after transplantation in autumn was as high as 80%, subsequent low temperatures induced dormancy of the plantlets.

Table 4. Shoot multiplication after 6 wk of culture, and elongation (shoots >1.5 cm) and rooting abilities after 25 d of culture of different *Davidia involucrata* individuals

Tree no.	Shoots per explant	Shoot elongation (%)	Rooting (%)	Tree no.	Shoots per explant	Shoot elongation (%)	Rooting (%)
1	2.6^{c}	33.3^{c}	75^{a}	**16**	1.5^{d}	3.3^{d}	-
2	3.6^{bc}	26.7^{c}	100^{a}	**17**	1.7^{d}	73.3^{b}	100^{a}
3	3.5^{bc}	33.3^{c}	100^{a}	**18**	1.5^{d}	73.3^{b}	80^{b}
4	4.8^{ab}	33.3^{c}	100^{a}	**19**	3.8^{b}	66.7^{b}	100^{a}
5	2.9^{c}	100.0^{a}	100^{a}	**20**	3.7^{bc}	100.0^{a}	100^{a}
6	6.6^{a}	73.3^{b}	100^{a}	**21**	2.7^{c}	100.0^{a}	100^{a}
7	4.3^{b}	100.0^{a}	100^{a}	**22**	5.4^{ab}	80.0^{b}	100^{a}
8	1.1^{e}	33.3^{c}	45^{c}	**23**	3.4^{bc}	100.0^{a}	100^{a}
9	2.7^{c}	33.3^{c}	35^{c}	**24**	3.2^{bc}	73.3^{b}	100^{a}
10	4.2^{b}	40.0^{bc}	100^{a}	**25**	1.8^{d}	73.3^{b}	75^{b}
11	3.7^{bc}	73.3^{b}	100^{a}	**26**	4.3^{b}	100.0^{a}	100^{a}
12	3.9^{b}	53.3^{bc}	100^{a}	**27**	4.6^{b}	100.0^{a}	100^{a}
13	2.9^{c}	33.3^{c}	10^{d}	**28**	6.5^{a}	93.3^{ab}	100^{a}
14	1.1^{e}	100.0^{a}	85^{b}	**29**	1.7^{d}	93.3^{ab}	75^{b}
15	6.1^{a}	100.0^{a}	100^{a}	**30**	4.3^{b}	100.0^{a}	100^{a}

Means in a column followed by the same letter do not significantly differ ($p < 0.05$) according to Duncan's test.

One year after being planted at 3 different locations, plantlets had similar average heights (5.2~5.9 cm) (Table 6). However, plantlets at Meifeng had the highest survival rate (97.6%), while that in the Taipei greenhouse was the lowest (43.6%). Since *D.*

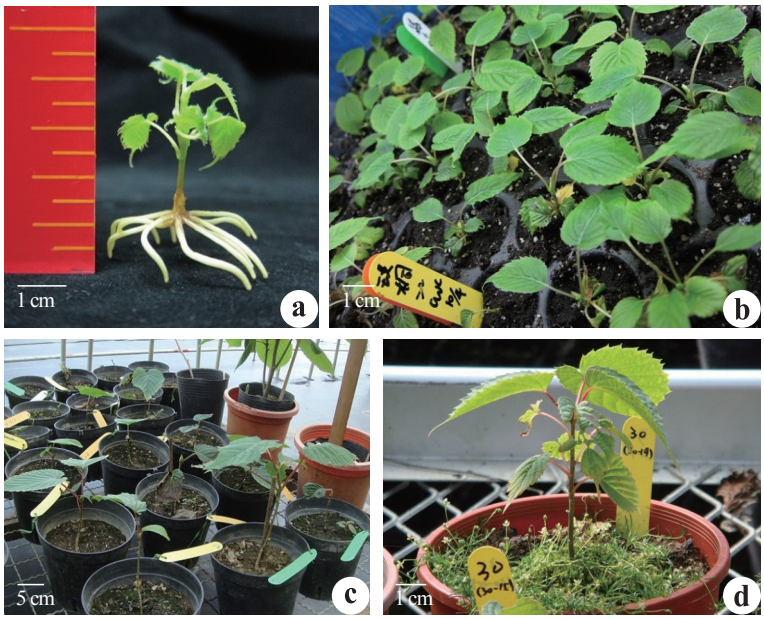

Fig. 4. Acclimation of *Davidia involucrata* plantlets. (a) A rooted plantlet from *in vitro* culture; (b) after 3 wk of acclimation in a growth chamber; (c, d) 1-yr-old plantlets cultured in greenhouses at the Lienhuachih Research Center (c) and at Meifeng Farm (d).

Table 5. Survival rates of transplanted plantlets cultured in a growth chamber for 3 wk and then transferred to a Taipei greenhouse for 1 mo in different months of 2011

Month	Growth chamber			Greenhouse	
	No. of initial plantlets	Surviving plantlets	Survival (%)	Surviving plantlets	Survival percentage based on plantlets that survived in the growth chamber
January	115	110	95.7	80	72.7
February	122	120	98.4	85	70.8
March	135	135	100.0	132	97.8
April	90	86	95.6	82	95.3
May	86	85	98.8	68	80.0
June	69	68	98.6	34	50.0
July	80	77	96.3	31	40.3
August	113	111	98.2	52	46.8
September	98	95	96.9	76	80.9
October	65	62	95.4	49	79.0
November	96	95	99.0	74	78.7
December	100	98	98.0	59	60.2

Table 6. Plantlet survival rates and growth after 1 yr of culture in greenhouses at Taipei Botanic Garden, the Lienhuachih Research Center, and Meifeng Farm

Location	Elevation (m)	Annual avg. temp (℃)	July avg. temp (℃)	July avg. highest temp (℃)	January avg. lowest temp (℃)	Total plantlets	Survival (%)	Range of plantlet height (cm)	Avg. plantlet height (cm)
Taipei Botanic Garden, TFRI	7	22.7[1)]	29.6[1)]	39.3[1)]	12.6[1)]	250	43.6	3~10.8	5.43±2.83
Lienhuachih Research Center[2)]	600[2)]	21.0[2)]	25.2[2)]	29.5[2)]	10.6[2)]	63	57.1	4~11.0	5.94±2.11
Meifeng Farm[3)]	2100[3)]	13.7[3)]	17.8[3)]	25.3[3)]	2.5[3)]	42	97.6	1~10.5	5.17±2.10

[1)] http://www.taipeitravel.net.

[2)] Lienhuachih Research Center, TFRI, Nantou County, Taiwan. http://www.tfri.gov.

[3)] Meifeng Farm, Highland Experimental Farm, National Taiwan University, Nantou County, Taiwan. http://mf.ntu.edu.tw.

involucrata grows naturally at mid-elevations (700~2500 m) with annual average temperatures of 8~12.4℃ (Zhang 1995), it cannot withstand hot temperatures (>38℃) (Zhan et al. 2010). The survival rate was the highest in Meifeng among all 3 sites, since it has the most suitable temperature and elevation for the growth of *D. involucrate.* The hot summer temperature in Taipei led to low survival rates in the Taipei greenhouse. Plantlets in Meifeng and Lienhuachih were defoliated and entered a dormant stage in winter, while 13.2% of the plantlets in Taipei still had leaves and continued growing in winter. This is the reason that the average height of plantlets in Taipei was almost the same as they were Meifeng and Lienhuachih. Overall, 1-yr-old plantlets did not grow well at any site. Plantlets sprouted only once (in spring) at Meifeng and Lienhuachih.

Table 7 shows plantlet growth of different individuals after 1 yr of culture at the 3 sites. In Taipei, tree no. 30 grew the best in terms of the average height and the number and percentage of plantlets with a height of >6 cm/10 cm. Among 33 surviving plantlets, 11 plantlets were >6 cm, including 3 at >10 cm. In contrast, tree nos. 4, 11, 13, and 15 grew the slowest with average heights of < 2 cm.

Plantlets at Lienhuachih could be divided into 2 groups: one, including tree nos. 3, 20, 22, 27, and 30, had higher average heights (of > 5.7 cm, Fig. 4c); the other, including tree nos. 15 and 24, had lower average heights (of 4 cm). At Meifeng, there were higher percentages of plantlets taller than 6 cm than at Taipei and Lienhuachih. Average heights of tree nos. 20, 27, and 30 at Meifeng Farm were >6 cm (Fig. 4d). Overall, tree no. 30 was the most adaptable individual in Taiwan, as its plantlets grew well in both the Taipei greenhouse (with an average high temperature in July of 39.3℃) and Meifeng (with an average low temperature in January of 2.5℃), followed by tree nos. 3, 20, 22, and 27.

CONCLUSIONS

In the present work, fully developed and unsprouted buds of *D. involucrata* were originally collected from Sichuan Province, China in late winter as micropropagation materials, and 29 of 30 individuals were successfully

Table 7. Heights of *Davidia involucrata* plantlets grown in greenhouses of 3 locations for 1 yr

Tree no.	Average plantlet height (cm)			No. of plantlets of >6 cm in height (%)/10 cm (%)		
	Taipei Botanic Garden, TFRI	Lienhuachih Research Center	Meifeng Farm	Taipei Botanic Garden, TFRI	Lienhuachih Research Center	Meifeng Farm
2	2.64±0.95	-	-	0/0	-/-	-/-
3	3.28±1.28	8.50±3.54	-	1 (8.3)/0	2 (100)/0	-/-
4	1.75±0.35	-	4.25±1.85	0/0	-/-	1 (25.0)/0
5	-	-	4.25±1.76	-/0	-/-	1 (33.3)/0
6	3.00±0.00	-	-	0/0	-/-	-/-
7	2.00±0.00	-	3.00±0.00	0/0	-/-	0/0
11	1.60±0.00	-	-	0/0	-/-	-/-
12	2.15±0.64	-	-	0/0	-/-	-/-
13	1.50±0.00	-	-	0/0	-/-	-/-
15	1.56±0.66	4.00±0.00	1.75±1.06	0/0	0/0	0/0
19	2.50±0.02	-	-	0/0	-/-	-/-
20	3.17±1.58	7.50±0.71	6.50±0.00	2 (11.8)/0	2 (100)/0	1 (100)/0
21	3.88±1.87	-	-	1 (25.0)/0	-/-	-/-
22	3.10±1.86	6.00±2.83	-	1 (10.0)/0	1 (50.0)/0	-/-
23	2.18±1.07	-	-	0/0	-/-	-/-
24	2.35±0.21	4.00±0.00	-	0/0	0/0	-/-
26	2.58±0.85	-	4.18±1.38	0/0	-/-	0/0
27	2.80±1.12	6.50±3.54	7.00±0.00	0/0	4 (66.7)/1(16.7)	1 (100)/0
28	2.45±0.40	-	4.50±0.00	0/0	-/-	0/0
30	4.91±2.77	5.75±1.41	6.04±1.99	11 (33.3)/3 (9.1)	2 (50.0)/0	11 (47.8)/2 (8.7)

propagated. Results showed great variations in performance of shoot proliferation, elongation, rooting, and growth in the greenhouse among different individuals. Micropropagated plantlets of those 29 individuals were transplanted to Taipei Botanic Garden greenhouse, and then portions were moved to greenhouses at Meifeng Farm and the Lienhuachih Research Center. At Meifeng, where the environment is similar to the natural habitat of *D. involucrata*, the plantlet survival rate was the highest, while that in Taipei was the lowest. Among individuals, tree no. 30 had the best growth performance at all 3 sites, followed by tree nos. 3, 20, 22, and 27. Generally, *D. involucrata* grew slowly in Taiwan. The average height was only 4 cm in the first year. To obtain more-comprehensive data in the future, we transplanted some plantlets at the Experimental Forests of National Taiwan Univ. and National Chung-Hsing Univ. to observe and record the adaptation of *D. involucrata* in Taiwan.

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