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TISSUE CULTURE AND PLANT REGENERATION OF *PRUNUS CAMPANULATA* MAXIM

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ABSTRACT

This study used the tender shoot of *Prunus campanulata* Maxim. of the current year as explants for the induction and rapid propagation of adventitious buds. The results showed that the bud induction rate peaked at 94.44% after treatment with Murashige and Skoog medium (MS) +2.0 mg L^{-1} 6-benzyladenine (6-BA) + 0.1 mg L^{-1} naphthalene acetic acid (NAA). The multiplication coefficient was 7.44 after treatment with MS +3.0 mg L^{-1} 6-BA + 0.01 mg L^{-1} NAA + 30 g L^{-1} sucrose. The combination of MS + 0.01 mg L^{-1} NAA + 0.3 mg L^{-1} gibberellic acid had the best growth-promoting effects (seedlings grew well; 2-4 cm high). The treatment of 1/2 MS + 1.0 mg L^{-1} indole-3-butyric acid + 0.5 mg L^{-1} IAA + 20 g L^{-1} sucrose had a rooting percentage of 82.35% in which the plants grew well with fast rooting and lengths of 3-4 cm. Treatment consisting of 1/2 MS +1.5 mg L^{-1} ABT one rooting powder (ABT_1) + 20 g L^{-1} sucrose had the highest rooting percentage (93.05%) and growth. The matrix of vermiculite and perlite (1:1) was optimal for transplantation with a survival rate of up to 94.07%. This study involved the optimal mediums and culture conditions, all of which were significant for industrialized breeding and seedling production.

Key words: *Prunus campanulata* Maxim., tissue culture, micropropagation

INTRODUCTION

The cherry blossom, a famous ornamental flower that is widely cultivated throughout the world, is the national flower of Japan and has cultivar groups of high ornamental value (Wang and Huang, 2001). In China, cherry blossom, mostly sakura (*Prunus serrulata*) but also Chinese cherry blossom, is commonly used in urban landscaping. The current development and utilization of Chinese *Cerasus* genus resources, especially wild native resources, cannot meet the urban landscaping demand. *Prunus campanulata* Maxim., known as Taiwan cherry, hilly cherry, and bellflower cherry, is a deciduous *Cerasus* tree belonging to the rose family (Rosaceae) that is native to Fujian, Zhejiang, Guangdong, Guangxi, and Taiwan. The flowering period of *Prunus campanulata* Maxim. bloom, which is featured in the yearly Spring Festival, can be up to 50 d (Chen *et al.*, 2011) , much longer than that of sakura (15 d) (Chen *et al.*, 2008).

Prunus campanulata Maxim has colorful flowers with a unique drooping bell-like shape, very high ornamental value, strong resistance, and wide adaptability, making it a rare native ornamental tree for landscaping (Lu *et al.*, 2006). Its wild population is in recession mode, and the regeneration of old trees plays an important role in its re-vegetation and natural regeneration (Lu *et al.*, 2006). *Prunus campanulata* Maxim. produces few seeds over a short collecting period that are often eaten by birds, its reproducing materials are scarce, and its propagation is mainly dominated by tender branch cutting with strict restrictions; thus it remains poorly understood, and the protection and utilization of its wild resources are far behind the actual needs and cannot meet the production demand (Lu *et al.*, 2006).

Researchers have investigated the features of *Prunus campanulata* Maxim., such as its community structure, ecological characteristics, population size, and fauna distribution (Lu *et al.*, 2006; Chen, 2007; Xu *et al.*, 2004; Wang *et al.*, 2006), clarified characteristics of its population structure and evolution, and performed studies on seedling breeding, asexual cuttings, and growth at the seedling stage (Fang, 2006; Kang, 2007; Zou *et al.*, 2008). Thus, the rapid propagation of *Prunus campanulata* Maxim using tissue culture techniques is an important seed breeding approach. A few reports are available on the in vitro propagation of *Prunus campanulata* Maxim., however, these research results had their own limitations, the multiplication coefficient of 3~4 shoots was inefficient and the study on rooting medium needed a heavy workload because of using too much kinds of hormones (Lu *et al.*, 2006; Huang *et al.*, 2006). In this study, Tissue culture techniques of *Prunus campanulata* Maxim. were studied systemly from initiation culture, proliferation culture, strong seedling culture, rooting culture, acclimatization and transplantation, superior individuals of *Prunus campanulata* Maxim. were utilized for *in vitro* culture to increase its propagation coefficient. We hope that the results of this study will provide valuable information for

the industrialized production of *Prunus campanulata* Maxim. plantlets.

MATERIALS AND METHODS

Plant materials and pretreatment: Explant materials were taken from *Prunus campanulata* Maxim. plus tree (FJ15) provided by Fujian Agriculture And Forestry University. Healthy pest-free *Prunus campanulata* Maxim. plants with tender shoots were selected as explants. The leaves and stipules at the tender tips were removed and the plants were rinsed with running water for 1 h, immersed in a 70% alcohol solution for 30-45 s for surface disinfection, rinsed again with sterile water three times, sterilized with 0.1% $HgCl_2$ for 5 min, and rinsed 4-6 more times for at least 2 min each. The remaining water was dried with filter paper and the samples were cut into 0.5-1.0 cm segments with buds for inoculation.

Methods

Culture medium and selection and optimization of inducing conditions: In the induction experiment, three basic culture media were used including Murashige and Skoog (MS), improved White's (WH), and improved MS. The 6-benzyladenine (6-BA; 1.0, 2.0, and 4.0 mg L^{-1}) and NAA (0.01, 0.1, and 1.0 mg L^{-1}) were selected with an orthogonal design (orthogonal array L9 (3^{3-1})). For each treatment, 30 culture bottles were inoculated with one explant each. Three replicates were designed. The bottles were observed every 5 days until the bud induction rate could be determined 25 days after the inoculation.

Selection and optimization of subculture media for propagation: MS was used as basic culture medium. In a L9 (3^{3-1}) orthogonal experiment, different concentrations of 6-BA (2.0, 3.0, and 4.0 mg L^{-1}), NAA (0.01, 0.1, and 0.5 mg L^{-1}), and sucrose (20, 30, and 40 g L^{-1}) were designed. For each treatment, 30 culture bottles were inoculated with two shoots each. Three replicates were designed. The bottles were observed every 5 days until the multiplication coefficient could be determined at 25 days after inoculation.

Selection and optimization of culture media for seedling growth promotion: MS was used as basic culture medium. Three treatments included 0.01 mg L^{-1} NAA + 0.1 mg L^{-1} gibberellic acid (GA_3), 0.1 mg L^{-1} NAA + 0.3 mg L^{-1} GA_3, and 0.5 mg L^{-1} GA_3. For each treatment, 30 culture bottles were inoculated with three shoots each. Three replicates were designed. The growth conditions were observed 25 days after inoculation.

Selection and optimization of culture media for rooting: To investigate the effects of basic media, indole-3-butyric acid (IBA), and IAA combinations on rooting, three basic media with 20 g L^{-1} sucrose addition (1/2 MS, woody plant medium, and improved WH), IBA (1.0, 2.0, 3.0 mg L^{-1}), and IAA (0.5, 1.0, and 1.5 mg L^{-1}) were used for a L9 (3^{3-1}) orthogonal experiment. For each treatment, 30 culture bottles were inoculated with three shoots each. Three replicates were designed. The rooting percentage was calculated 25 days after inoculation.

To investigate the effects of ABT_1 (ABT one rooting powder) and ABT_3 (ABT three rooting powder) on rooting, 1/2 MS was used as basic culture medium and ABT_1 (0.5, 1.5, and 3.0 mg L^{-1}) and ABT_3 (0.5, 1.5, and 3.0 mg L^{-1}) were used. For each treatment, 30 culture bottles were inoculated with three shoots each. Three replicates were designed. The rooting percentage was calculated 25 days after inoculation.

Transplantation of regenerated plants: After the test-tube plantlet took roots, the culture bottle was opened and moved to a transitional culture room for acclimation. The plantlet was collected and the culture medium was rinsed off of its roots. The cleaned plantlet was transplanted to a matrix sterilized with 0.1% potassium permanganate. The matrix was composed of sand (E1), garden soil (E2), and a vermiculite and perlite mixture (E3) in equal proportions. There were three treatments with thee replicates each. The survival rate of the plantlets over the 30 days after transplantation was calculated.

RESULTS AND DISCUSSION

i. Effects of induction media on the induction of segments with buds: The effects of the basic culture media (MS, improved WH, and improved MS) as well as growth regulator 6-BA and NAA on the induction of segments with buds were studied. All of the treatments could induce multiple shoots and had good induction effects. Table 1 shows the ranges of the induction rates for the three factors. The induction rates were highest for basic media, lowest for IAA, and in the midrange for 6-BA. The variance analysis revealed a significant (P = 0.0093 and 0.0154) difference in induction rates for basic media and 6-BA but no difference (P = 0.5410) in that for IAA. The findings indicated that the induction rates of the buds could be affected by basic culture media and changes in 6-BA concentration but not by changes in IAA concentration. The analysis of the ranges of induction rates suggested that the mean induction rate of the buds peaked at 94.44%, and bud quality was high for $_1$6-BA_2IAA_2 (the treatment of MS + 2.0 mg L^{-1} 6-BA + 0.1 mg L^{-1} IAA). Therefore, MS + 2.0 mg L^{-1} 6-BA + 0.1 mg L^{-1} IAA was the optimal medium for bud induction.

Table 1. Effect of different basic medium and types of plant growth regulators on stem budding index

		Factors			Budding rate (%)
		Basic culture medium	6-BA	NAA	
Treatment	1	MS	1.0	0.01	58.33
	2	MS	2.0	0.1	94.44
	3	MS	4.0	1.0	76.19
	4	Improved WH	1.0	0.1	17.39
	5	Improved WH	2.0	1.0	50.00
	6	Improved WH	4.0	0.01	23.81
	7	Improved MS	1.0	0.01	36.36
	8	Improved MS	2.0	0.1	73.68
	9	Improved MS	4.0	1.0	56.25
Average Budding rate	T1	75.32	36.36	50.94	
	T2	29.40	71.71	55.03	
	T3	54.43	51.08	53.18	
	R	45.92	35.35	4.09	

Ti（i=1,2,3） expresses the average of the corresponding levels. *R* shows the difference between maximum and minimum. The same below.

ii. Effects of subculture conditions on bud proliferation: The explants inducted for 4 weeks in induction media were cultured in MS subculture medium. After 4 weeks, a large number of stretched adventitious buds were found and used for the proliferation induction. The results (Table 2) showed that among the nine treatments, the multiplication coefficient in the treatment of MS + 3.0 mg L^{-1} 6-BA + 0.01 mg L^{-1} NAA + 30 g L^{-1} sucrose peaked at 7.44. Meanwhile, the T values revealed that this treatment was optimal for the induction of bud proliferation. Analysis of the multiplication coefficient ranges indicated that bud proliferation could be most affected by 6-BA, followed by NAA and then sucrose. Variance analysis revealed that the multiplication coefficient was significantly (P = 0.0063 and 0.0414) affected by 6-BA and NAA concentration but not (P = 0.2515) by sucrose concentration. The bud proliferations in the subculture media are shown in Figure 1.

Table. 2 Effect of growth regulators and the mixture on bud subculture proliferation

		Factors			25d multiplication coefficient
		6-BA	NAA	Sucrose	
Treatment	1	2.0	0.01	20	3.5
	2	2.0	0.1	30	3.09
	3	2.0	0.5	40	2.67
	4	3.0	0.01	40	7.44
	5	3.0	0.1	20	6.25
	6	3.0	0.5	30	5.11
	7	4.0	0.01	30	3.36
	8	4.0	0.1	40	2.33
	9	4.0	0.5	20	1.92
Mean average multiplication coefficient	T1	3.09	4.77	3.65	
	T2	6.27	3.89	4.15	
	T3	2.54	3.23	4.09	
	R	3.73	1.53	0.5	

Fig.1 Bud proliferations in the subculture media of *Prunus campanulata* Maxim.

iii. Effect of growth regulators on seedling cultivation: The shoots derived from subculture were short, small, and slim with less obvious caulom, short internodes, low lignification level, and heights < 1.5 cm. Further seedling culture was conducted to improve the rooting percentage. After 25 days, the rootless seedling growth was greatly improved. The treatment of MS + 0.1 mg L^{-1} NAA + 0.3 mg L^{-1} GA_3 had the best growth-promoting effects in which the seedling had a height of 2-4 cm and increased diameter and grew well (Table 3). Figure 2 shows the growth of the seedlings in growth-promoting culture.

Table 3. Effects of growth-promoting culture

Treatment	Growth
NAA 0.01 mg L^{-1} + GA_3 0.1 mg L^{-1}	Normal growth, height of 1-2 cm, slow to elongate
NAA 0.1 mg L^{-1} + GA_3 0.3 mg L^{-1}	Good growth, height of 2-4 cm, thick stems
GA_3 0.5 mg L^{-1}	Slim shoots, height of 3–5 cm

Fig. 2. Growth of the seedlings in growth-promoting culture of *Prunus campanulata* Maxim.

iv. Effects of basic culture medium, growth regulator, ABT_1, and ABT_3 on rooting: The effects of basic culture medium and the combination of different concentrations of growth regulators on rooting percentage of *Prunus campanulata* Maxim. were obvious. Analysis of the rooting percentage range suggested that it could be most affected by IBA, followed by IAA and then basic culture medium. Analysis of variance (ANOVA) showed that the rooting percentage was significantly ($P < 0.01$) affected by basic culture medium as well as concentrations of IBA and IAA. The healthy twigs of seedlings that were approximately 1.5 cm high were used for rooting in the culture medium. In a week, the roots appeared. In 25 days, the roots reached lengths up to 1 cm (Table 4). Among the treatments, the combination of MS + 0.1 mg L^{-1} IBA + 0.5 mg L^{-1} IAA + 20 g L^{-1} sucrose had the highest rooting percentage of 82.35% and the roots grew well and fast with a length of approximately 3 cm.

Different concentrations of ABT_1 and ABT_3 could induce rooting (Table 5). Among all treatments, the combination of 1/2 MS + 1.5 mg L^{-1} ABT_1 + 20 g L^{-1} sucrose had the highest ($P < 0.05$) rooting percentage of 93.05% in which the seedlings grew well. Within 0.5-3.0 mg L^{-1}, the rooting percentage increased and then decreased with increasing ABT_1 concentrations and the rooting percentage was highest after treatment with 1.5 mg $L^{-1}ABT_1$; however, in contrast with the ABT_1 treatment results, the rooting percentage increased with increasing ABT_1 concentrations and the rooting percentage was highest after treatment with 3.0 mg L^{-1} ABT_3. However, the roots grew poorly after treatment with 3.0 mg L^{-1} ABT_3. The rooting in culture media is shown in Figure 3.

v. Transplantion of regenerated plants: The appropriate planting matrix is an important factor for transplantation survival. Table 6 shows that the different matrix had obvious effects on the survival rates of the transplanted seedlings. ANOVA revealed a significant ($F = 854.67$, $P = 0.0001$) influence of the different matrix on the survival rate of the transplanted plants. The least significant difference test for multiple comparisons revealed that E3 (vermiculite:perlite = 1:1) was the optimal matrix for the transplantation of plants with an average survival rate of 94.07%, followed by E2 (garden soil) with an average survival rate of 59.12%. The survival rate of plants after E3 treatment was significantly different from those of the other two treatments.

Table 4. The effect of different medium and types of plant growth regulators on rooting

		Factors			Rooting %	Growth
		Basic culture medium	IBA	IAA		
Treatment	1	1/2 MS	1.0	0.5	82.35	Grew well and fast, rooted fast, sturdy roots, 4-5 roots, root length approx. 3 cm
	2	1/2 MS	2.0	1.0	62.50	Grew less well, little callus, thick roots, 2-3 roots, root length approx. 2 cm
	3	1/2 MS	3.0	1.5	44.67	Grew poorly, more callus, slow rooting, root length approx. 1 cm
	4	WPM	1.0	1.0	70.83	Grew well, little callus, fast rooting, 5-7 roots, root length approx. 1.5 cm
	5	WPM	2.0	1.5	32.00	Grew poorly and slowly, more callus, slow rooting
	6	WPM	3.0	0.5	48.46	Grew less well, more callus, slow rooting, slim and thin roots, root length < 1 cm
	7	Improved WH	1.0	1.5	51.81	Grew less well, slow rooting, sturdy roots, root length approx. 1 cm
	8	Improved WH	2.0	1.0	43.69	Grew less well, more callus, slim and thin roots, root length approx. 2.5 cm
	9	Improved WH	3.0	0.5	46.82	Grew poorly, more callus, slow rooting, slim roots, 8-9 roots, root length approx. 1 cm
Mean average rooting percentage	T1	63.17	68.33	58.17		
	T2	50.43	46.06	60.05		
	T3	47.44	46.65	42.83		
	R	15.73	22.27	17.22		

Table 5. Effect of the different concentration of ABT_1 and ABT_3 on rooting culture

Treatment	Rooting percentage (%)	Growth
1/2 MS + ABT_1 0.5 mg L^{-1}	52.28 e	Grew less well, robust roots, 3-4 roots, root length approx. 3 cm
1/2 MS + ABT_1 1.5 mg L^{-1}	93.05 a	Grew well, robust roots, 5-6 roots, root length approx. 3 cm
1/2 MS+ ABT_1 3.0 mg L^{-1}	60.46 d	Grew poorly, slim roots, 5-6 roots, root length approx. 2 cm
1/2 MS + ABT_3 0.5 mg L^{-1}	46.09 f	Grew less well, robust roots, 4-5 roots, root length approx. 3 cm
1/2 MS + ABT_3 1.5 mg L^{-1}	66.22 c	Grew well, robust roots, 5-6 roots, root length approx. 2 cm
1/2 MS + ABT_3 3.0 mg L^{-1}	71.30 b	Grew poor, slim roots, 8-9 roots, root length approx. 1.5 cm

Different letters in the columns show significant difference at $P_{0.05}$ by Duncan's multiple test. The same below.

Fig.3 Rooting in culture media of *Prunus campanulata* Maxim.

Table 6. Effect of different substrate on the survival rate of transplants

Treatment	Survival rate (%)
Sand	47.37 c
garden soil	59.12 b
vermiculite:perlite	94.07 a

Discussion and conclusion: Cultivating winter- resistant varieties for cold northern regions is an important *Prunus campanulata* Maxim. breeding approach. The main cultivation methods include selection from native *Prunus campanulata* Maxim. and inducing cold-resistance genes into *Prunus campanulata* Maxim. by transgenic breeding technology. Regardless of conventional breeding or molecular breeding techniques, a complete in vitro rapid propagation system is a must for technical support. Therefore, the study of tissue culture of *Prunus campanulata* Maxim. is of significance for industrialized breeding and seedling production.

In plant tissue culture, the rational use of growth regulators to control plant cell and tissue growth, differentiation, organogenesis, embryogenesis, and regeneration of whole plants is important (Al-Maarri *et al.*, 1994). In this study, the basic medium and 6-BA concentrations had great impacts on the bud induction rate, and the optimal induction medium for *Prunus*

campanulata Maxim. was MS + 2.0 mg L^{-1} 6-BA + 0.1 mg L^{-1} NAA with the highest bud induction rate of 94.44%. In the subculture test, the medium of MS + 3.0 mg L^{-1} 6-BA + 0.1 mg L^{-1} NAA + 30 g L^{-1} sucrose was optimal for bud proliferation with an effective multiplication coefficient up to 7.44. The seedling obtained from subculture was short and weak with less effective buds. The culture medium was further adjusted and the results showed that treatment with MS + 0.1 mg L^{-1} NAA + 0.3 mg L^{-1} GA_3 resulted in well-growing seedlings with heights of 2-4 cm and thick stems. In the rooting test, the treatment with 1/2 MS basic culture medium had better rooting effects on *Prunus campanulata* Maxim. The 1/2 MS added with different hormones (IBA and IAA) and rooting powder (ABT_1 and ABT_3) could induce rooting. Treatment with 1/2 MS + 1.5 mg L^{-1} ABT_1 + 20 g L^{-1} sucrose had the highest rooting percentage (93.05%) and good plant growth. Compared with previous studies, rooting cycles was shortened by 10 d and rooting medium was opitimized without reducing rooting percentage (Lu *et al.*, 2006). This study involved bud proliferation culture, growth-promoting culture, and rooting induction culture, all of which were keys to the successful development of a tissue culture system for *Prunus campanulata* Maxim.

During the tissue culture of *Prunus campanulata* Maxim., the culture bottles were required to be open for acclimation prior to transplantation and required high humidity, low light levels, proper ventilation, and low temperatures at the start of culture. The plantlets were transplanted 6 days later. The survival rate of the seedling was greatly affected by the different matrixes. The mixed matrix of vermiculite and perlite in equal proportions was less contaminated, easy to disinfect, good for ventilation and water retention, and could provide an optimal environment for plantlet growth, thereby improving the survival rates of the transplanted seedling with an average survival rate of 94.07%.

REFERENCES

Al-Maarri, K.,Y. Arnaud and E. Miginiac (1994). Micropropagation of pyrus communis cultivar ‘passe crassane’ seedlings and cultivar ‘williams’: factors affecting root formation in vitro and ex vitro. *Scientia* Horticulturae, *58*:207–214.

Chen, Z. (2007). Quantitative characteristics and floristic analysis of species in wild community of *Prunus campanulata* Maxim. J. Fujian Forestry Science and Technology. 34(9):48-51.

Chen, Z., Y. L. Lu and W. X. Wu (2011). Study on ornamental characteristics and floral biological characteristics of *Prunus campanulata* Maxim. Fujian Science & Technology of Tropical Crops. 36(3):1-5.

Chen, Z. H., M. Xiao and X. Chen (2008). Change in flowering dates of Japanese cherry blossoms (*P. yedoensis* Mats.)in Wuhan University campus and its relationship with variability of winter temperature. Acta Ecologica Sinica. 28(11): 5209-5217.

Fang M. H. (2006). The study on the cuttage grafting technology for *Prunus campanulata* Maxim. J. Fujian Agricultural Science and Technology. 4:40-41.

Huang, Y. X., J. Y. Liu, X. L. Zhuo and Z. F. Wang (2006). A Study on Tissue Culture of *Prunus campanulata* Maxim.. Chinese Agricultural Science Bulletin. 28(3):162-164.

Kang, M. S. (2007). Seed Development Observation and seedling Raising Experiment of *Prunus campanulata* Maxim. J. Fujian Forestry Science and Technology. 34(3):19-22.

Lu, Y. L., J. S. Shi, Z. Chen, C. D. Liu and X. R. Liu (2006). The Study on Community Characteristics of *Prunus campanulata* Maxim. J. Fujian Forestry Science and Technology. 33(2):29-33.

Lu, Y. L., Z. Chen and J. S. Shi (2006). Research Advance, Prospect and Breeding Strategy of *Prunus campanulata* Maxim. J. Nanjing Forestry University (Natural Sciences Edition). 30(1):115-118.

Lu, Y.L., Z. Chen, J. S. Shi, Y. X. Huang, J. Y. Liu and J. L. Xie (2006). Adventitious Bud Inducing and Plant Regeneration of *Cerasus campanulata* Maxim. in Large Scale. J. Nanjing Forestry Uni. (Natural Sciences Edition). 30(3):105-108.

Wang X. R., D. L. Yan, N. G. Yi (2006). Characteristics of the *Prunus campanulata* Maxim. Community Structure and Its Species Diversity. J. Southwest Forestry College. 26(4):9-13.

Wang, X. R., G. F. Huang (2001). The development and utilization of the plant resources Chinese cherry. *Forestry science and technology*.15(6), 3-6.

Xu, K. F., X. R. Wang, D. L. Yan and X. G. Yi (2004). Ecological Characteristics of *Prunus campanulata* Maxim. J. Nanjing Forestry Uni. (Natural Sciences Edition). 28 (6): 111-114.

Zou, N., N. Xu, G. Q. Cao, S. Z. Lin and Q. L. Lin (2008). Optimization of Rooting Condition of *Prunus campanulata* Maxim. Cuvette Plantlet. Jiangxi J. Agricultural Sciences. 20(4):26-29.