Pak. J. Bot., 48(1): 305-309, 2016.

OPTIMIZING THE RAPID TECHNIQUE FOR PROPAGATION OF *CERASUS CAMPANULATA* BY TISSUE CULTURE

BIHUA CHEN[1,2*], JIANMIN LI[1,2], JUAN ZHANG[1,2], ZHUOXI WU[1,2], HUIHUA FAN[1,2] AND QIANZHEN LI[1,2]

[1]*Fujian Academy of Forestry Sciences, Fuzhou 350012, China;*
[2]*Key Laboratory of Timber Forest Breeding and Cultivation for Mountainous Areas in Southern China, China Forestry Bureau, Fuzhou 350012, China*
[*]*Corresponding author's e-mail: chenbihua.happy@163.com*

Abstract

The initiation medium for *Cerasus campanulata* was 1/2MS+1.0 mg L^{-1} BA+0.1 mg L^{-1} NAA +30 g L^{-1} sugar, and the average initiation rate was 94.4%. The proliferation medium composed of MS+0.3 mg L^{-1} BA+0.2 mg L^{-1} NAA+30 g L^{-1} sugar provided proliferation rate 3.6 and average shoot length 2.3 cm. The rooting medium composed of 1/2MS+0.6 mg L^{-1} IBA+20 g L^{-1} sugar provided rooting rate 85.6%, average root number per individual 5.9 roots, and average root length 2.7 cm. The dishwashing detergent instead of Tween-20 as the spreader reduced the contamination rate for the explant disinfection. The red-core soil adopted to be the plantlet transfer medium provided 91.3% survival rate. The light media LZ and SK were not eligible to be the transfer medium for the species.

Key words: *Cerasus campanulata* (Maxim.) Yu et Li, Tissue culture; Medium, Dishwashing detergent; red-core soil.

Introduction

Cerasus campanulata (Maxim.) Yu et Li (previous name *Prunus campanulata* Maxim), namely Fujian cherry blossom, belongs to genus *Cerasus*, family Rosaceae. The species is a deciduous tree flowering in winter and early spring lasting for 2-3 months in China. The petals are red catering to Chinese favor. This species is native to Fujian and Taiwan China and differs from Japanese cherry blossom. The wild cherry blossom in China comprises 48 species. *C. campanulata* is widely applied as a greening tree in gardens and on roadside.

C. campanulata in nature differs in petal color, flower number per cluster and bloom time, therefore the individual sales price are different. The traditional propagation for *C. campanulata* is by seeds, thus the seedling (i.e. progeny) characteristics divers and some of the excellent characteristics can not be inherited. Although the tissue culture techniques of *C. campanulata* (Huang, 2014; Huang *et al*., 2006; Jia, 2010; Lv *et al*., 2006; Wang & Huang, 2002; Xu, 2008; Zou & Lin, 2013; Zou *et al*., 2008; Zou *et al*., 2013) and its relative *Cerasus serrulata* (Huang *et al*., 2003; Li, 2013; Li *et al*., 2014), *Prunus subluta* (Wang *et al*., 2004), Ornamental cherry (Wang & Li, 2006), *Laurocerasus caroliniana* (Su *et al*., 2008) and *Prunus serrulata* 'Royal Burgundy' (Li *et al*., 2008) have been reported, but they have different results and can not achieve mass propagation. Our purpose is to optimize the propagation technique of *C. campanulata* by tissue culture and set up an industrial production system to provide plenty of *C. campanulata* seedlings for the human demand. The excellent trees of *C. campanulata* were selected to study the tissue culture. The seedlings were mass propagated and inherited their fine characteristics.

Materials and Methods

The sprouts from the trunk base of elite individuals of *C. campanulata* in Laizhou National Forestry Farm were used as the plant material. The apical buds and hemi-lignified stems were adopted as the explants.

Surface sterilization: The stems were initially washed under running tap water for 20 min, cut into pieces with 2-3 nodes each, immersed in 70% ethanol for 60 seconds under aseptic condition in a laminar flow machine, rinsed in sterilized water once, transferred to 0.1% $HgCl_2$ supplemented with Tween-20 or dishwashing detergent for 15 min, and then rinsed in sterilized water four to five times. For surface sterilization 100 ml 0.1% $HgCl_2$ was supplemented with 2 drops of Tween-20 or dishwashing detergent respectively for the contrastive test. The explants were cut two ends with one node each (length 1.5-2.0 cm) then transferred onto shoot initiation medium.

Culture conditions: The explants were incubated in dark for the first 2 weeks, followed with 500-1000 lux illumination. The illumination intensity was 1000-1500 lux for shoot proliferation and root induction, and 3000-6000 lux for plantlet hardening provided with 12-h light photoperiod. All the cultures were maintained in room temperature of 24±2°Ċ.

Experimental design

Explant initiation: The explant initiation medium comprised: (1) MS+1.0 mg L^{-1} BA+0.1 mg L^{-1} NAA; (2) 1/2MS+1.0 mg L^{-1} BA+0.1 mg L^{-1} NAA. All media contained 30 g L^{-1} sugar and 6.0 g L^{-1} carrageenan (produced in Quanzhou, Fujian, China), pH 6.0. There were 50 jars for each medium and 3 replication for the same experiment. One bud or shoot occupied one jar respectively. The data of initiation rate and contamination rate were recorded after 30-day incubation.

Shoot multiplication: The shoot proliferation medium comprised: (3) MS+1.0 mg L^{-1} BA+0.1 mg L^{-1} NAA; (4) 1/2MS+1.0 mg L^{-1} BA+0.1 mg L^{-1} NAA; (5) MS+0.3 mg L^{-1} BA+0.2 mg L^{-1} NAA; (6) 1/2MS+0.3 mg L^{-1} BA+0.2 mg L^{-1} NAA. All media contained 30 g L^{-1} sugar and 6.0 g L^{-1} carrageenan, pH 6.0. There were 30 jars (3 shoots per jar) for each medium and 3 replication for the same experiment. The data of proliferation rate and shoot length were recorded after 30-day incubation.

Root induction: The rooting medium comprised: (7) 1/2MS+1.0 mg L^{-1} NAA+0.2 mg L^{-1} IBA+30 g L^{-1}sugar (Huang *et al*., 2006; Huang, 2014; Lv *et al*., 2006); (8) 1/2MS+0.6 mg L^{-1} IBA+15 g L^{-1} sugar (Zou *et al*., 2008; Zou *et al*., 2013; Zou & Lin, 2013); (9) 1/2MS+1.0 mg L^{-1} NAA+1.0 mg L^{-1} IBA+0.75 mg L^{-1} BA+15 g L^{-1} sugar (Wang & Huang, 2002); (10) 1/2MS+0.4 mg L^{-1} IBA+15 g L^{-1} sugar; (11)1/2MS+0.6 mg L^{-1} IBA+20 g L^{-1} sugar. All media were solidified with 6.0 g L^{-1} carrageenan, pH 6.0. There were 30 jars (3 shoots per jar) for each medium and 3 replication for the same experiment.

Plantlet hardening: After 20-day culture in rooting medium in culture room, the plantlets with the bottles were transferred into the glass greenhouse for 15-day hardening. Rooting percentage, root number per individual, root length and plantlet length were recorded at the end of 15-day hardening.

Plantlet transplant: The red-core soil (natural local soil) or light materials were adopted as the cultivation media. The soil or media was put into the black plastic bags with drain holes, placed in the greenhouse, and sterilized by 0.03%-0.05% $KMnO_4$. The hardened plantlets were rinsed by tap water to eliminate the remains of carrageenan. The plantlets were immersed in 1/1000 dilution of 70% Thiophanate methyl or 1/1000 dilution of 80% Mancozeb solution for 10 min, then transplanted into the soil or light media and covered with a transparent plastic film upon arched racks to maintain high humidity. The plantlets were watered once every day. The plantlets were sprayed by 1/800 dilution of 70% Thiophanate methyl or 1/800 dilution of 80% Mancozeb solution to control the diseases every week. The film was disclosed after 2 weeks and the watering frequency increased to 2-3 times per day. The data of survival rate were recorded after 60 days.

Statistical analysis: Data were processed statistically with SPSS 17.0 software. Data were performed by analysis of variance (ANOVA) (for 3-6 means) or *t*-test (for 2 means), with a post-hoc *Tukey*'s test if the ANOVA was significant. Means are provided with standard errors, and means were considered significantly different at $p<0.05$.

Results

Spreader for explant sterilization: For surface sterilization 100 ml 0.1% $HgCl_2$ was supplemented with 2 drops of Tween-20 or dishwashing detergent respectively for the contrastive test. The results are shown on Table 1.

From the view of Table 1, there is no significant difference between Tween-20 and the control, but significant difference between dishwashing detergent and Tween-20 or dishwashing detergent and the control. The contamination rate is the lowest by using dishwashing detergent. The dishwashing detergent is the optimal spreader for explant sterilization.

Explant initiation medium: The test of explant initiation medium is shown on Table 2. The results showed no significant difference between medium No. 1 and 2. New shoots on medium No. 1 appeared over length shoots, oversize leaves and partial curly leaves. New shoots on medium No. 2 grew vigorously. Therefore, medium No. 2: 1/2MS+1.0 mg L^{-1} BA+0.1 mg L^{-1} NAA was optimal for explant initiation of *C. campanulata*.

Table 1. The effect of Tween-20 and dishwashing detergent on the explant sterilization

Disinfectant	Spreader	Average contamination rate (%)
$HgCl_2$	Tween-20	8.16±0.134 a
$HgCl_2$	Dishwashing detergent	5.77±0.189 b
$HgCl_2$	(Control)	7.69±0.078 a

± Shows value of standard deviation from treatment mean. Different letter(s) after data within a column represent statistically significant difference among treatment means at $p\leq0.05$ using *Tukey* test

Proliferation medium: Under aseptic conditions, the elongating shoots were excised and placed on the different proliferation media for three successive subcultures in same medium. The results (Table 3) showed the proliferation rates on medium No. 3 and 4 were the highest of all, 5.5 and 5.1, respectively. There are no significant differences between medium No. 3 and 4 on proliferation rate and average shoot length, respectively. The average shoot length on medium No. 3 and 4 were short, 1.3 cm and 0.9 cm, respectively, unqualified for root induction. The average proliferation rate on medium No. 5 was 3.6, although it was lower than that of medium No. 3 and 4, its shoot length achieved 1.8 cm, qualified for root induction. The indexes on medium No. 6 were inferior to that of No. 5. Therefore, medium No. 5: MS+0.3 mg L^{-1} BA + 0.2 mg L^{-1} NAA was optimal for shoot proliferation of *C. campanulata* (Fig. 1).

Rooting medium: The elongating shoots were excised and placed on rooting media. After 20-day incubation in culture room, the plantlets with bottles were transferred into the glass greenhouse for 15-day hardening. The roots were recorded and analyzed on Table 4. The results showed the average rooting rate was highest on medium No. 11 providing 85.67% rooting. The order of average rooting rate from high to low: medium No. 11 > No. 8 > No. 10 > No. 9 > No. 7. The order of average root number per individual from high to low: medium No. 7 > No. 10 > No. 8, 9, 11. Although the average root number per individual was only 5.9 roots on medium No. 11, it reached the qualified standard. The order of average root length from high to low: medium No. 7, 8, 11 ≥ No. 7, 10 > No. 9. Also the average root length achieved 2.7 cm on medium No. 11, reaching the qualified standard. Therefore, only medium No. 11 with double "a" level was optimal to be the rooting medium for *C. campanulata* (Figs. 2, 3).

Table 2. The effect of different basal media on explant initiation of *C. campanulata*.

Medium No.	Medium	BA (mg L^{-1})	NAA (mg L^{-1})	Average initiation rate (%)	Growth status
1	MS	1.0	0.1	91.9±1.25 a	Over length shoots, oversize leaves and partial curve leaves
2	1/2MS	1.0	0.1	94.4±1.25 a	Vigorous shoots and leaves

± Shows value of standard deviation from treatment mean. Different letter(s) after data within a column represent statistically significant difference among treatment means at $p \leq 0.05$ using *t*-test

Table 3. The effect of different media on shoot proliferation of *C. campanulata*.

Medium No.	Medium	BA (mg L^{-1})	NAA (mg L^{-1})	Average proliferation rate	Average shoot length (cm)	Growth status
3	MS	1.0	0.1	5.5 ± 0.15 a	1.3 ± 0.06 c	Too many new buds; not elongating shoots; green leaves; oversize old leaves
4	1/2MS	1.0	0.1	5.1 ± 0.10 a	0.9 ± 0.10 c	Too many new buds; not elongating shoots; yellowing and undersize old leaves
5	MS	0.3	0.2	3.6 ± 0.15 b	2.3 ± 0.10 a	Optimal amount new buds; elongating shoots; green leaves; optimal size leaves
6	1/2MS	0.3	0.2	2.9 ± 0.06 c	1.8 ± 0.10 b	Optimal amount new buds; elongating shoots; yellowing leaves

± Shows value of standard deviation from treatment mean. Different letter(s) after data within a column represent statistically significant difference among treatment means at $p \leq 0.05$ using *Tukey* test.

Table 4. The effect of different media on root induction of *C. campanulata*.

Medium No.	Medium	NAA (mg L^{-1})	IBA (mg L^{-1})	BA (mg L^{-1})	Sugar (g L^{-1})	Average rooting rate (%)	Average root number per individual (root)	Average root length (cm)	Growth status
7	1/2MS	1.0	0.2	0	30	57.4±0.36 e	10.9±0.30 a	2.4±0.15 ab	Partial yellowing leaves; not elongating shoots
8	1/2MS	0	0.6	0	15	79.5±0.35 b	6.1±0.10 c	2.8±0.06 a	Partial yellowing leaves; elongating shoots
9	1/2MS	1.0	1.0	0.75	15	62.2±0.55 d	5.2±0.12 c	1.6±0.10 c	Partial yellowing leaves; not elongating shoots
10	1/2MS	0	0.4	0	15	75.6±0.60 c	8.8±0.38 b	2.2±0.15 b	Partial yellowing leaves; slow growth
11	1/2MS	0	0.6	0	20	85.6±0.47 a	5.9±0.31 c	2.7±0.06 ab	Green leaves; vigorous growth

± Shows value of standard deviation from treatment mean. Different letter(s) after data within a column represent statistically significant difference among treatment means at $p \leq 0.05$ using *Tukey* test.

Table 5. The effect of conversion media on the survival rate of *C. campanulata*.

Medium No.	Medium	Average survival rate (%)	Growth status
1	Light medium LZ	4.4 ± 0.55 c	The survived seedlings grew slowly; partial leaves turned yellowing
2	Light medium SK	67.9 ± 0.63 b	The survived seedlings grew slowly; partial leaves turned yellowing
3	Red-core soil	91.3 ± 1.15 a	The survived seedlings grew vigorously

± Shows value of standard deviation from treatment mean. Different letter(s) after data within a column represent statistically significant difference among treatment means at $p \leq 0.05$ using *Tukey* test

Plantlet transplant: Light medium LZ comprised: peat + husk of rice + used medium by mushroom (main ingredient: saw dust) + slow release fertilizer. Light medium SK comprised: peat + bark + saw dust + slow release fertilizer. The two light media were compared to red-core soil on plantlet survival rate. The data are shown on Table 5. The results indicated the survival rate was very low in light media LZ, providing 4.4% survival rate only, and the survived seedlings did not grow vigorously. The survival rate was not high in light media SK, providing 67.9% survival rate only, and the survived seedlings did not grow vigorously as well. The survival rate was satisfied in red-core soil, providing 91.3% survival rate, and the survived seedlings grew vigorously. There were significant difference among the soil, LZ and SK media. Therefore, the red-core soil was optimal for plantlets conversion (Figs. 4, 5, 6).

Discussion

The optimal initiation medium for *C. campanulata* is 1/2MS+1.0 mg L^{-1} BA+0.1 mg L^{-1} NAA+30 g L^{-1} sugar, providing 94.4% initiation rate. The basal medium 1/2MS is the same to that of Xu (2008) and Jia (2010), but the plant growth regulators are different as well as its concentration. Jia (2010) used 20 g L^{-1} sugar instead of 30 g L^{-1}. Huang *et al*. (2006), Lv *et al*. (2006) and Wang & Huang (2002) adopted 1/4MS and Huang (2014) adopted MS instead of 1/2MS as the basal medium. Wang & Rong (2008), Zou & Lin (2013) and Zou *et al*. (2013) propagated the species by callus culture, and the media they applied are very different. The full-strength inorganic MS is harmful to the explants of *C. campanulata*.

Fig. 1. Proliferation buds of *C. campanulata*.

Fig. 4. Eligible plantlets of *C. campanulata* for transplant.

Fig. 2. Rooted plantlets of *C. campanulata*.

Fig. 5. Transplanted plantlets of *C. campanulata*.

Fig. 3. Hardening plantlets of *C. campanulata*.

Fig. 6. Six-month-old seedlings of *C. campanulata*.

The optimal proliferation medium for *C. campanulata* is MS+0.3 mg L^{-1} BA+0.2 mg L^{-1} NAA+30 g L^{-1} sugar, providing 3.6 fold proliferation rate and 2.3 cm shoot length. The basal medium MS is the same to that of Huang *et al*. (2006), Jia (2010), Lv *et al*. (2006), Wang & Huang (2002) and Xu (2008), but they supplemented GA_3 in the media. Huang (2014) adopted 3/2MS as the basal medium not supplemented with GA_3. The half-strength inorganic MS is lack of nutrient to the shoot proliferation and elongation of *C. campanulata*.

The optimal rooting medium for *C. campanulata* is 1/2MS+0.6 mg L^{-1} IBA+20 g L^{-1} sugar, providing 59 roots per individual and 2.7 cm root length. The basal medium 1/2MS is the same to that of Huang *et al*. (2006), Jia (2010), Lv *et al*. (2006), Wang & Huang (2002), Xu (2008), Zou & Lin (2013) and Zou *et al*. (2013), but the plant growth regulators are different as well as its concentration. Jia (2010) used 15 g L^{-1} sugar instead of 20 g L^{-1}, which caused partial leaves yellowing. Huang (2014) adopted 1/3MS as the basal medium, the inorganic salts of which is lower and different from others.

This paper first reports the dishwashing detergent is the optimal spreader for explant sterilization. The detergent is easily obtained from a supermarket and reduces the explant contamination rate effectively. Its price is much cheaper than Tween-20.

The red-core soil was optimal for plantlets conversion, providing 91.3% survival rate. It is cheap and easy to obtain because of local soil. The two light media are not eligible for transplanting the plantlets of *C. campanulata*, since the survival rate was 4.4% or 67.9% only. The fertilizer in the light media may cause the plantlet dying out while acclimatizing to the new environment. Another defect is the light medium has not water retention, and the long time mist wastes plenty of water, thus it can not be used in the drought area. As the transplant medium, Wang & Huang (2002) adopted perlite; Huang *et al*. (2006) and Lv *et al*. (2006) adopted perlite and peat; Huang (2014) adopted perlite, peat and red-core soil; Xu (2008) adopted red-core soil and vermiculite. The survival rate from Xu (2008) was 76.0% only. The merit of red-core soil is easy to disinfect. The used medium by mushroom containing much fungus or bacteria is not suitable for plantlets conversion.

The researchers have reported the tissue culture techniques of relative species, such as *Cerasus serrulata* (Huang *et al*., 2003; Li, 2013; Li, 2014), *Prunus subluta* (Wang *et al*., 2004), *Laurocerasus caroliniana* (Su *et al*., 2008) and *Prunus serrulata* 'Royal Burgundy' (Li *et al*., 2008). The media vary at different stages since they are different species.

Acknowledgements

The research was funded by China 2014 Forestry Public Welfare Research Project (Project No. 201404715).

References

Huang, S. 2014. The experiment on tissue culture of *Cerasus campanulata* Maxim. *J. Fujian Forest. Sci. & Technol.*, 41(2): 103-106.

Huang, S., J. Chi, S. Su, W. Shang and Y. Ren. 2003. Tissue culture and rapid propagation of *Prunus serrulata* in Wuling Mountain. *Plant Physiol. Comm.*, 39(3): 228.

Huang, Y., J. Liu, X. Zhuo and Z. Wan. 2006. A study on tissue culture of *Prunus campanulata* Maxim. *Chinese Agri. Sci. Bull.*, 22(8): 162-164.

Jia, L. 2010. Studies on adventitious bud culture and somatic embryogenesis of *Cerasus campanulata*. Master thesis, Nanjing Forestry University, Nanjing, China.

Li, M. 2013. Ecological characteristics and tissue culture of the high altitude population of *Cerasus serrulata*. Master thesis, Nanjing Forestry University, Nanjing, China.

Li, X., X. Lu, Z. Bian and L. Long. 2014. Study on rapid propagation of *Cerasus serrulata* by tissue culture. *Forest. Sci. & Technol*., 39(5): 1-3.

Li, Y., Y. Meng, X. Zhao, H. Wang, Q. Zhang and L. Wang. 2008. Tissue culture and rapid propagation of *Prunus serrulata* Lindl. "Royal Burgundy". *Plant Physiol. Comm.*, 44(6): 1163-1164.

Lv, Y., Z. Chen, J. Shi, Y. Huang and J. Xie. 2006. Adventitious bud inducing and plant regeneration of *Cerasus campanulata* Maxim. in Large Scale. *J. Nanjing Forest. Uni.,* (*Natural Science Edition*), 30(3): 105-108.

Su, J., G. Tang, G. Wang and Y. Liu. 2008. Tissue culture and plant regeneration of *Laurocerasus caroliniana* Ait. *J. Nanjing Forest. Uni.,* (*Natural Science Edition*), 32(4): 98-100.

Wang, G. and M. Huang. 2002. Tissue culture and plant regeneration of *Cerasus campanulata*. *J. Nanjing Forest. Uni.,* (*Natural Science Edition*), 26(02): 73-76.

Wang, W. and X. Li. 2006. Tissue culture of ornamental cherries flower stems. *J. Anhui Agri. Sci.*, 34(22): 5839-5841.

Wang, X. and D. Rong. 2008. Application of orthogonal design in tissue culture of *Cerasus campanulata* (Rosaceae). *J. Anhui Agri. Uni.*, 35(2): 169-173.

Wang, Y., J. Xu, J. Luo, Y. Shi and X. Liu. 2004. Study on the technique of tissue culture and rapid propagation of *P. subulata* L. *Shandong Agri. Sci.*, (6): 6-8.

Xu, N. 2008. Study on tissue culture technique of *Prunus campanulata.* Master thesis, Fujian Agriculture and Forestry University, Fuzhou, China.

Zou, N. and S. Lin. 2013. Plant regeneration and somatic embryogenesis of *Prunus campanulata*. A*dvances in ornamental Horticulture of China*, 285-291.

Zou, N., N. Xu, G. Cao, S. Lin and Q. Lin. 2008. Optimization of rooting condition of *Prunus campanulata* Cuvette Plantlet. *Acta Agri. Jiangxi*, 20(4): 26-29.

Zou, N., Z. Chen, S. Lin and Q. Lin. 2013. Callus induction and plant regeneration of *Prunus campanulata*. *J. Nuclear Agri. Sci.*, 27(10): 1417-1423.

(Received for publication 26 February 2015)