Germplasm preservation *in vitro* of *Polygonum multiflorum* Thunb

He-Ping Huang[1,3], Jian Wang[1], Lu-Qi Huang[3], Shan-Lin Gao[2], Peng Huang[1], Dian-Lei Wang[1]

[1]Anhui Academy of Traditional Chinese Medicine, Anhui University of Traditional Chinese Medicine, Hefei, Anhui, [2]Department of Genetics and Breeding, China Pharmaceutical University, Nanjing, Jiangsu, [3]Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, People's Republic of China

Submitted: 23-09-2012 Revised: 27-09-2012 Published: 17-04-2014

ABSTRACT

Background: The root of *Polygonum multiflorum* Thunb. is a common traditional Chinese medicine. In recent years, the wild resources of *P. multiflorum* have been seriously broken, and the cultivated varieties have been degrading. The germplasm resources of *P. multiflorum* need protection and preservation. So far, no *in vitro* germplasm preservation of *P. multiflorum* has been reported. **Objective:** To explore a method for the *in vitro* germplasm preservation of *P. multiflorum*. **Materials and Methods:** A large number of buds from seed explants were induced by tissue culture. The single buds were used as experimental materials to study the effects of plant growth regulator, temperature, and osmotic pressure on the preservation time, growth recovery, and genetic stability. **Results:** When the buds were inoculated onto Murashige and Skoog (MS) basal media containing 4% w/v sucrose, 2% w/v mannitol, and 1% w/v sorbitol, supplemented with paclobutrazol (PP_{333}) 1.0 mg/l, abscisic acid (ABA) 5.0 mg/l, and daminozide (B9) 30.0 mg/l in an illuminated chamber under a 16 h photoperiod of 1500 lx light intensity at 15°C for 10 months, the survival rate was over 70% with good growth recovery and genetic stability. **Conclusion:** The results of this study can be used for medium-term *in vitro* germplasm preservation of *P. multiflorum*, and meeting actual needs of research and production.

Key words: Germplasm, *Polygonum multiflorum* Thunb., preservation *in vitro*

Access this article online
Website: www.phcog.com
DOI: 10.4103/0973-1296.131032
Quick Response Code:

INTRODUCTION

Polygonum multiflorum Thunb., of the genus *P. lapathifolium*, is a perennial twisted herbal plant.[1] The root is a common traditional Chinese medicine. In addition, the stem and leaf of *P. multiflorum* can be also used as medicinal material.[2] In clinical practice, raw root was used for detoxification, elimination carbuncle, and catharsis.[3-5] After processing, it was used for nourishing liver and kidney, enriching blood, and blackening hair.[3-5] In the recent years, with the trend of returning to nature and the improvement of people's living standards, the demand for natural herbs have surged. Owing to the increasing market demand, the wild resources of *P. multiflorum* are being seriously broken.[6,7] Besides, the cultivated varieties of *P. multiflorum* have been degrading in the process of long-term cultivation, which resulted in reducing the yield and quality of medicinal material.[8] Germplasm resources of *P. multiflorum* are being destroyed. Consequently, it is necessary to protect and preserve germplasm resources of *P. multiflorum*. However, there still has been no report on the germplasm preservation of *P. multiflorum*. To preserve germplasm preservation of *P. multiflorum,* we sought here a protocol for *in vitro* germplasm preservation of *P. multiflorum*.

Address for correspondence:
Prof. Jian Wang, Anhui Academy of Traditional Chinese Medicine, Anhui University of Traditional Chinese Medicine, Hefei, Anhui-230038, People's Republic of China.
E-mail: hhp760222@126.com

Germplasm resources are the material basis of breeding.[9] Preservation of plant germplasm is significant for the conservation of biodiversity and preventing the loss of good gene. Research into germplasm preservation on crops was frequently reported.[10-13] Nevertheless, studies on germplasm preservation of medicinal plants were focused on preservation *in vitro*.[14-16]

With the seeds or roots used as propagation materials, *P. multiflorum* can only maintain genetic stability in short time. Because of frequently natural hybridization, the genetic basis presents high heterogeneity, which leads to the instability of good character in sexual reproduction. And asexual reproduction is susceptible to the virus with germplasm degradation.[8] Preservation *in vitro* based on

tissue culture, not only can effectively preserve germplasm of *P. multiflorum* but also can rapidly propagate to meet production needs. To the best of our knowledge, no *in vitro* germplasm preservation of *P. multiflorum* has been reported. In this paper, an *in vitro* preservation protocol for *P. multiflorum* has been established and optimized. Buds from seed explants were induced by tissue culture. Buds *in vitro* were preserved for 10 months under room temperature (15°C), and the survival rate was over 70% with good growth recovery and genetic stability. The methods will provide a scientific basis for the germplasm preservation as well as further varieties breeding of *P. multiflorum*.

MATERIALS AND METHODS

Plant material

Seeds of *P. multiflorum* ($2x = 22$) were obtained from Qianshan County, Anhui Province, China. The Department of Genetics and Breeding of China Pharmaceutical University identified the original plant.

Seed disinfection and germination and culture conditions

Seeds of *P. multiflorum* were sterilized in 2% v/v sodium hypochlorite containing three to five drops/l of Tween-20 for 12 min. The seeds were rinsed in sterile distilled water three to five times and then transferred to a petridish containing sterile filter paper to remove excess surface water. The sterilized seeds were placed onto Murashige and Skoog (MS) (Murashige and Skoog 1962) medium containing 3% w/v sucrose and 0.33% w/v agar powder (gel strength = 1,100 g/cm^2) at pH 5.8. The inoculated seeds were incubated in an illuminated chamber under a 16 h photoperiod of 1,200 lx light intensity at 25 ± 1°C to initiate germination.

Experiment on effects of plant growth regulators by orthogonal test

MS medium was the basal medium used throughout these studies. To extend *in vitro* preservation time of plant material, three phytohormones, namely, paclobutrazol (PP_{333}; 0.5, 1.0, and 2.0 mg/l), abscisic acid (ABA; 1.0, 5.0, and 10.0 mg/l), and daminozide (B9; 10.0, 20.0, and 30.0 mg/l) were used at three concentrations each for the orthogonal test. The experimental plant materials were robust single-buds with five single-buds inoculated for each 150 ml conical flask. In this experiment, the nine treatments defined above were inoculated into six flasks each. Preservation time with materials survival rate of not less than 70% was taken as evaluation standards.

Screening for culture temperature

Buds inoculated on breeding medium supplemented with 0.8 mg/l 6-benzylaminopurine (BAP), 0.2 mg/l α-naphthaleneacetic acid (NAA) were cultured at 0, 5, 15, and 25°C, respectively. In this experiment, the four treatments defined above were inoculated into ten 150 ml conical flasks each with five single-buds inoculated for each flask. To obtain an objective evaluation about the effects of the culture temperature, the growth state of buds and start time of buds wilt were also observed. The evaluation standards were identical to the former.

Experiment on effects of osmotic pressure by orthogonal test

Single-buds were selected to inoculate onto MS medium supplemented with penetrants. In this experiment, three penetrants, namely, sucrose (20, 40, and 60 g/l), mannitol (5, 10, and 20 g/l), and sorbitol (5, 10, and 20 g/l) were used at three concentrations each for the orthogonal test. Not only were five single-buds inoculated into each 150 ml conical flask, but also the nine treatments defined above were inoculated into 10 flasks each. The inoculated buds were incubated in an illuminated chamber under a 16 h photoperiod of 1,500 lx light intensity at 25 ± 1°C. Evaluation standards were the same as the former.

Growth recovery of materials preserved *in vitro*

The survival materials, preserved for 300 days by the optimal preservation method, were subcultured on the breeding media supplemented with 0.8 mg/l BAP and 0.2 mg/l NAA under a 16 h photoperiod of 1,200 lx light intensity at 25 ± 1°C. After they have been subcultured for three 25-day subculture cycle, the propagation coefficients, leaf length, and leaf width of survival materials were observed. Furthermore, after three 25-day subculture cycle, survival materials were transferred to rooting media consisting of semi-solid MS media at 1/2 macronutrient concentration and supplemented with 0.3 mg/l NAA to induce roots for observing rooting rate, as well as subsequent chromosome determination. In our experiment, the control materials routinely subcultured with 25-day subculture cycle on breeding media supplemented with 0.8 mg/l BAP, 0.2 mg/l NAA, were also incubated in an illuminated chamber under a 16 h photoperiod of 1,500 lx light intensity at 25 ± 1°C.

Genetic stability determination

Root tips approximately 0.5 cm in length were excised and pretreated in a 0.2% w/v colchicine solution for 2 h. The root tips were fixed in Carnoy's fluid (containing 3:1 ethanol and glacial acetic acid) at 3-5°C for 2-24 h, rinsed with 95% (*w/v*) alcohol, 70% (*w/v*) alcohol, and distilled water three times, respectively, and then macerated for 16 min with 0.2 M HCl at 60°C. After being soaked in distilled water for 40 min, the fixed root tips were stained with improved Carbol Fuchsin (1.8 g sorbitol dissolved in 10 ml Carbol Fuchsin, then mixed with 45% v/v acetic acid 90 ml). A photomicroscope (Olympus BX 40, Japan) was used for chromosome determination.

RESULTS AND DISCUSSION

Effects of plant growth regulators on preservation *in vitro*

For many plant species, growth retardants applied to the medium of preservation *in vitro* are necessary to inhibit growth and delay aging. Paclobutrazol (PP_{333}), a plant growth inhibitor, works by blocking the oxidation of kaurene to kaurane, different acids inhibit the biosynthesis of plant gibberellin (GA) to delay the growth of plant.[17,18] Studies have demonstrated that PP_{333} played an important role in preservation *in vitro*.[19] From the 1960s, the discovery and identification of ABA,[20] the physiological functions of ABA, such as inhibition of plant cell elongation and division, induction of seed dormancy, and prevention of premature germination, have been constantly revealed.[21] Daminozide (B9), a plant growth retardant, can inhibit the biosynthesis of the plant endogenous hormones to play an important role in regulating plant growth, and promoting strong seedling.[22,23]

In our research, the orthogonal test revealed that the variation of the PP_{333} concentration (2,379.00), and the variation of the ABA concentration (900.33) had significant effects on the preservation time [Table 1]. Further analysis showed that the longest preservation time was 133.33 days found at PP_{333} concentration of 1.0 mg/l, and 121.33 days found at ABA concentration of 5.0 mg/l, respectively [Table 2]. The effect of B9 on the preservation time was not significant. According to these results, we may draw a conclusion that the best preservation media for buds *in vitro* of *P. multiflorum* were the MS media supplemented 1.0 mg/l PP_{333}, 5.0 mg/l ABA, and 30.0 mg/l B9.

Furthermore, in our experiment, preservation time of three treatments with survival rate of over 70% was over four months (data not presented). Moreover, the survival materials can normally grow in breeding medium supplemented with 0.8 mg/l BAP, 0.2 mg/l NAA at 25°C.

The *K*-value is the sum of the preservation time of all tests with the same factor at the same level and the *R*-value is the difference between the maximum and minimum value of *K* with the same factor. The *K*-values and the effects of each level with the same factor are positive correlation. *R*-values and the effects of each factor are positive correlation.

Effects of temperature on preservation *in vitro*

Room-temperature preservation and cryopreservation are two methods of germplasm preservation *in vitro*. Cryotemperature is suitable for long-term preservation,[24] while room-temperature is often used for medium-short-term preservation. Compared with cryopreservation, room-temperature preservation without special equipment is more convenient and cheaper.

In preservation *in vitro*, appropriate temperature is key to extending the preservation time. Different plants and different genotypes of the same plant have different sensitivity of temperature.[24] It was believed that 0-6°C was suitable for temperate plants to preserve, whlie 15-25°C was suitable for tropical plants.[25] *P. multiflorum* is a widely distributed species in China.[1] In our experiment, when buds were preserved in 0°C, 5°C, 15°C, and 25°C, respectively, the preservation time were 211, 192, 173, and 62 days, and the start time of wilt were 188, 169, 154, and 47 days [Table 3]. With the temperature dropping, the growth of materials slowed down until it stopped. Compared with 15°C, when materials were preserved in 0 and 5°C, the prolongation of preservation time was limited, but the equipment conditions and energy consumption correspondingly increased. Because the materials rapidly grew in 25°C, preservation time (62 days) could not meet the objective of medium-short-term preservation *in vitro*. Therefore, we believed 15°C was the most appropriate temperature to medium-short-term preservation *in vitro* in our experiment.

When survival materials kept for six months at 15°C were subcultured into breeding media supplemented with 0.8 mg/l BAP, 0.2 mg/l NAA in an illuminated chamber under a 16 h photoperiod of 1500 lx light intensity at 25°C,

Table 1: Variance analysis of the preservation time of *P. multiflorum in vitro* buds by an orthogonal test

Source of variance	Sum of variance squares	Degree of freedom	Variance	*F* value	*P* value
PP_{333}	4,758.00	2	2,379.00	63.73*	0.01<*P*<0.05
ABA	1,800.67	2	900.33	24.12*	0.01<*P*<0.05
B9	98.67	2	49.33	1.32	*P*>0.1
E (error)	74.67	2	37.33	1.00	
Sum	6,732.01	8			

F1-0.01 (2,2)=99.0, F1-0.05 (2,2)=19.0, F1-0.1 (2,2)=9.0, *Significant at *P*=0.05

Table 2: Visual analysis of the preservation time of *P. multiflorum in vitro* buds by an orthogonal test

Concentration of phytohormone (mg/l)			Factor		
PP_{333}	ABA	B9	A (PP_{333})	B (ABA)	C (B9)
0.5	1.0	10.0	$K_A^{1/3}$=90.33	$K_B^{1/3}$=91.00	$K_C^{1/3}$=103.33
1.0	5.0	20.0	$K_A^{2/3}$=133.33	$K_B^{2/3}$=121.33	$K_C^{2/3}$=96.67
2.0	10.0	30.0	$K_A^{3/3}$=80.33	$K_B^{3/3}$=91.67	$K_C^{3/3}$=104.00
R (range)			53.00	30.33	7.33

materials were able to restore growth in 12 days. Then, room temperature (15°C) *in vitro* preservation of buds of *P. multiflorum* could meet the needs of production with a large number of propagations in short time.

Preservation time was the period with material survival rate of not less than 70%. Start time of wilt was the period from inoculation to the start of browning and death. Growth state was observed with naked eye.

Effects of osmotic pressure on preservation *in vitro*

Increase of medium osmotic pressure can inhibit the materials' *in vitro* growth to extend the preservation time. When the negative osmotic potential of the medium was increased to result in water stress, cell would difficultly absorb water to diminish metabolism, slow down growth and simultaneously, enzyme activity inhibition retard the cell wall growth to reduced nutrient consumption.[19,26] That medium was supplemented with carbohydrate, such as sucrose, mannitol, and sorbitol, and was a common method to improve the osmotic pressure.[27-30]

Sucrose, mannitol, and sorbitol are of hypertonic compounds, which can increase the osmotic pressure of the medium to prevent water absorption and decrease metabolic activity of cell. The orthogonal test revealed that the variation of the sucrose concentration (525.78), and the variation of the mannitol concentration (360.44) had significant effects on the preservation time [Table 4]. Further analysis showed that the longest preservation time was 82.00 days found at sucrose concentration of 40 g/l, and 75.33 days found at mannitol concentration of 20 g/l, respectively [Table 5]. The effect of sorbitol on the preservation time was not significant. According to these results, we may draw a conclusion that the MS medium containing 4% w/v sucrose, 2% w/v mannitol, and 1% w/v sorbitol was most suitable *in vitro* preservation medium for buds of *P. multiflorum*.

The results of our experiment were in accord with the conclusion that hypertonic compounds can delay the growth of plant reported in many literatures.[29-31] Moreover, The carbohydrate not only adjust the medium osmotic pressure, but also was the main carbon source for the plantlets growth *in vitro*.

The *K*-value is the sum of the preservation of all tests with the same factor at the same level and the *R*-value is the difference between the maximum and minimum value of *K* with the same factor. The *K*-values and the effects of each level with the same factor are positive correlation. *R*-values and the effects of each factor are positive correlation.

Growth recovery and chromosome determination

Preservation *in vitro* is an important means for the preservation of plant germplasm resources.[32] The preservation time was extended while the genetic stability of materials was maintained, these were the purpose of germplasm preservation *in vitro*.[33] According to our experimental results, *in vitro* buds were inoculated onto MS basal media containing 4% w/v sucrose, 2% w/v mannitol, and 1% w/v sorbitol supplemented with PP_{333} 1.0 mg/l, ABA 5.0 mg/l, and B9 30.0 mg/l in an illuminated chamber under a 16 h photoperiod of 1500 lx light intensity at 15°C. After 10 months, buds survival rate was over 70%. When the survival materials were inoculated onto the breeding media supplemented with 0.8 mg/l BAP, 0.2 mg/l NAA at 25°C, after three 25-day subcultures, multiplication time of buds, leaf length, and leaf width, rooting rate of survival materials and control materials were not significantly different. The multiplication time of buds of survival materials was 14.21, while that of the control materials

Table 3: The results of the experiment in different preservation temperature

Temperature/d °C	Preservation time/d	Start time of wilt/d	Growth state
0	211	188	Buds stopped growing
5	192	169	Buds grew very slow
15	173	154	Buds grew slowly
25	62	47	Buds grew rapidly

Table 4: Variance analysis of the preservation time of *P. multiflorum in vitro* buds by an orthogonal test

Source of variance	Sum of variance squares	Degree of freedom	Variance	*F* value	*P* value
Sucrose	1,051.56	2	525.78	45.96*	0.01<*P*<0.05
Mannitol	720.89	2	360.44	31.51*	0.01<*P*<0.05
Sorbitol	37.56	2	18.78	1.64	*P*>0.1
E (error)	22.89	2	11.44	1.00	
Sum	1,832.90	8			

F1-0.01 (2,2)=99.0, F1-0.05 (2,2)=19.0, F1-0.1 (2,2)=9.0, *Significant at *P*=0.05

Table 5: Visual analysis of the preservation time of *P. multiflorum in vitro* buds by an orthogonal test

Concentration of phytohormone (g/l)			Factor		
Sucrose	Mannitol	Sorbitol	A (Sucrose)	B (Mannitol)	C (Sorbitol)
20	5	5	$K_A^{1/3}$=62.67	$K_B^{1/3}$=54.67	$K_C^{1/3}$=65.67
40	10	10	$K_A^{2/3}$=82.00	$K_B^{2/3}$=71.33	$K_C^{2/3}$=70.00
60	20	20	$K_A^{3/3}$=56.67	$K_B^{3/3}$=75.33	$K_C^{3/3}$=65.67
R (range)			25.33	20.66	4.33

was 15.67. The leaf length and leave width of survival materials were about 9.2 mm and 8.3 mm, respectively, while the control materials were about 9.6 mm and 8.2 mm. The rooting rate of the survival materials was 93%, while the control material was 91% [Table 6].

In our experiment, the morphological appearance mutation of survival materials *in vitro* did not occur as compared with the control materials. Microscopic studies confirmed the chromosome number of survival materials to be 22 which was consistent with the literatures [Figure 1].[34,35] Compared with the control materials, the chromosome morphology of survival materials appeared normal. According to the growth recovery, the number and morphology of chromosome of the survival materials, buds *in vitro* of *P. multiflorum* had a good genetic stability in our experiment.

Leaves were chosen from each of 10 survival materials and each of 10 control materials. Ten stomata were measured for each leaf. Number of samples used for rooting is both 100.

In summary, under a 16 h photoperiod of 1500 lx light intensity, when *in vitro* single-buds of *P. multiflorum* inoculated onto MS media containing 4% w/v sucrose, 2% w/v mannitol, and 1% w/v sorbitol supplemented with PP_{333} 1.0 mg/l, ABA 5.0 mg/l, and B9 30.0 mg/l were preserved for 10 months in an illuminated chamber at 15°C, survival rate was not less than 70%. Moreover, the survivals had a good growth recovery and genetic stability.

Table 6: The growth recovery of the survival materials *in vitro*

Material	Times of buds multiplication	Leaf length (mm)	Leaf width (mm)	Rooting rate (%)
Control materials	15.67	8.2	9.6	93
Preservation materials	14.21	8.3	9.2	91

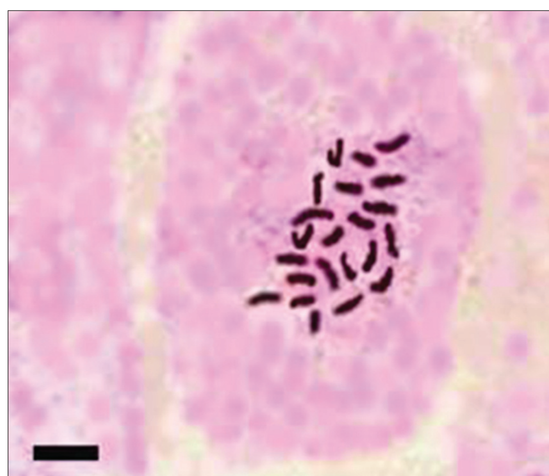

Figure 1: The chromosome of preservation materials *in vitro* (2n=2×=22; bar: 5.18 × 10-4 cm)

REFERENCES

1. Fu LG, Chen TQ, Lang KY, Hong T, Ling Q. Higher plants of China. Vol. 4. Qingdao: Qingdao Publishing House; 2000. p. 491.
2. Zhang L, T KL, Liao HM, Hu ZH. Content changes of stilbene glucoside in *Polygonum multiflorum* Thunb. Acta Bot Boreal Occident Sin 2010;30:1481-4.
3. Dong JX, Jia AL, Dong XL, Qiu ZD. Extraction process of anthraquinones in Radix Polygoni multiflori. J Changchun Univ TCM 2012;28:150-1.
4. Liu ZL, Li LF, Song ZQ, Wang C, Zhang L, Chao ZM. Chemical constituents from Radix Polygoni multiflori Thunb. After preparing. Nat Prod Res Dev 2009; 21:239-41.
5. Shi C, Zhang LC, Zhao RH, Zhang RP. The Chemical composition and identification of processing *Polygonum multiflorum*. J Kunming Med Univ 2011;32:45-7.
6. Tian S, Pu XF. Breeding and cultivation of *Polygonum multiflorum*. Spec Eco Anim Plant 2002;5:31.
7. Liu YH, Wang LH, Cao FL, Wei YL, Wei L X. Research advance in the high-yielding cultivation technique of *Polygonum multiflorum* Thunb. J Anhui Agric Sci 2007;35:5486,5511.
8. Long Y, Yang X, Yu C X. Studies on tissue culture of *Polygonum multiflorum* Thunb. Guid J TCM 2005;11:63,72.
9. Huang YX. The exchange, utilization and protection of rice germplasm. Modern Agric Sci Tech 2010;18:76-7.
10. Xu Y, Chen JH, Luan MB, Wang XF, Sun ZM. Research progress on conservation techniques for Ramie germplasm resources. J Plant Gen Resour 2011;12:184-9.
11. Tan ML, Yan MF, Wang L, Wang LJ, Yan XC. Status of special oil crops germplasm conservation in the world. J Plant Gen Resour 2011;12:339-45.
12. Zheng GH, Jing XM, Tao KL. Ultradry seed storage cuts cost of gene bank. Nature 1998;393:223-4.
13. Vertucci CW, Roos EE. Theoretical basis of protocols for seed storage. Plant Physiol 1990;94:1019-23.
14. Zhang XL, Liu WY, Zhang N, Zhou CY, Li MJ. Effect of PP_{333} on conservation *in vitro* of *Rehmannia glutinosa f. hueichingensis* (Chan et Sehih) Hsiao plantlets. J Henan Normal Univ (Natural Science) 2009;37:171-4.
15. Gu DZ, Gao HD, Lu S, Feng Y. Screening of media for *in vitro* culture and germplasm conservation *in vitro* of *Ophioglossum thermale* Kom. J Zhejiang Univ (Science Edition) 2011;38:205-10.
16. Fu CM, Zhao ZG, Huang ZL, Li F, Tang FL. Preservation *in vitro* of medicinal plant Salvia prionitis. Guihaia 2007;27:653-7.
17. Feng F, Ye CH, Guo JY, Li FQ. Effects of plant growth inhibitors on plantlets *in vitro Dioscorea alata* L. Crops 2007;2:29-31.
18. Gao FJ, Tang ZM, Wang XL, Qi SJ. Application progress of P_{333} used in plant tissue culture. Agric Tech 2002;22:66-9.
19. Huang HP, Wang J, Gao SL, Hang P. Study on preservation germplasm of *Dioscorea zingiberensis* C.H. Wright *in vitro*. J Chin Med Mater 2011;34:680-3.
20. Addicott FT, Carns HR. History and introduction. In: Addicott FT, editor. Abscisic Acid. New York: Praeger Sci; 1983. p. 1-21.
21. Cui KR, Xing GS, Zhou GK, Liu XM, Wang YF. The induced and regulatory effects of plant hormones in somatic embryogenesis. Hereditas 2000;22:349-54.

22. Huang P, Ma CH, Yan Q. Effect of plant growth retardant B9 on growth of test-tube plantlets of potato. Southwest Chin J Agric Sci 2011;24:1719-21.
23. Cui C, Wang JC, He FF, Zhou QY, Liu XZ, Li WB. Influences of the composition of MS medium and B9 concentration on the growth of virus-free potato plantlets in *in vitro* culture. J Southwest Agric Univ 2001;23:414-7.
24. Xu GB. Plant germplasm conservation *in vitro*. J Central South Forestry Univ 2000;20:81-7.
25. Omura M, Hidak T. Shoot tip culture of citrus II: Longevity of culture shoots. Bull Fruit Tree Res Stat 1992,22:33-7.
26. Dong LY, Yan JQ. Effects of 6-B A and mannitol on the cell expansion growth and the activities of cell wall-bound enzymes in cucumber cotyledons. Plant Physiol Commun 1992;28:47-50.
27. Divakaran M, Nirmal BM, Peter KV. Conservation of Vanilla species, *in vitro*. Sci Hort 2006;110:175-80.
28. Hao YJ, Deng XX. Cytological and molecular evaluation of strawberry plants recovered from *in vitro* conservation by slow-growth. J Hortic Sci Biotech 2005;80:588-92.
29. Westcott RJ. Tissue culture storage of potato germplasm: Use of growth retardants. Potato Res 1981;24:343-52.
30. Withers LA, Wheelans SK, Williams JT. *In vitro* conservation of crop germplasm and the IBPGR databases. Euphytica 1990;45:9-22.
31. Gopal J, Anjali C, Debabrata S. *In vitro* production of microtubers for conservation of potato germplasm: Effect of genotype, abscisic acid, and sucrose. *In vitro* Cell Dev Biol Plant 2004;40:485-90.
32. Martin KP, Pradeep AK. Simple strategy for the *In vitro* conservation of Ipsea malabarica an endemic and endangered orchid of the Western Ghats of Kerala, India. Plant Cell Tissue Organ Cult 2003;74:197-200.
33. Hong XR, Li MJ. Cryopreservation technique of Dioscorea opposita germplasm by vitrification. Chin Tradit Herb Drugs 2006;37:1715-8.
34. Liu CL. Chromosome analysis of *Polygonum multiflorum* Thunb. J Liaocheng Teachers Univ (Natural Science Edition) 1998;11:79-81.
35. Zhang BB, Zhang L, Wang HG. Cytology of *Polygonum mutiflorum* Thunb. Chin J Cell Biol 2006;28:112-4.

Cite this article as: Huang H, Wang J, Huang L, Gao S, Huang P, Wang D. Germplasm preservation *in vitro* of *Polygonum multiflorum* Thunb. Phcog Mag 2014;10:179-84.

Source of Support: This work was supported by the Natural Science Fund of Anhui University of Traditional Chinese Medicine (2010zr011B), the Natural Science Fund of Education Department of Anhui Province, China (KJ2011A191), and the Special Fund of Major Projects of National Science and Technology (2009ZX09301-005-03-03), **Conflict of Interest:** None declared.