Zhang et al., J Plant Physiol Pathol 2018, 6:5
DOI: 10.4172/2329-955X.1000190

Journal of Plant Physiology & Pathology

Research Article

A SCITECHNOL JOURNAL

Technological System Construction on Artificial Embryos Synchronization in Artificial Seeds Production of *Pinellia ternata* (Thunb.) Breit

Ming-Sheng Zhang*, Huan Li, Ye Hang, Gui-Xian Liu and Xiang Lv

Abstract

Using *Pinellia ternata* leaf blade or petiole as explants, the single-factor tests and orthogonal tests were used to research the effects from different explants and plant growth regulators to callus induction, cell micromasses suspension, cell micromass expansion and artificial embryo formation of *P. ternata*. Results showed that all induced calluses grew well and became looser using leaf blade explants and the combination of 2.0 mg/L 2,4-D and 1.5 mg/L BA or using petiole explants with 1.5 mg/L 2,4-D and 1.5 mg/L BA in the induction medium. The loose callus after three subculture with 2.0 mg/L 2,4-D and 1.5 mg/L 6-BA in medium was suitable materials to use as cell micromasses expansion culture. The expanded cell micromasses were differentiated and developed to form well synchronizing artificial embryo by suspension culture (suspension medium with 1.0 mg/L 2,4-D, 0.5 mg/L 6-BA, 40 g/L sucrose and 300 mg/L CH) and differentiation culture (differentiation medium with 0.5 mg/L 6-BA, 0.05 mg/L IBA, 10 g/L sucrose and 300 mg/L CH). This study successfully constructed and optimized the technology system of artificial embryos synchronization culture of *P. ternate*, it realized a breakthrough of the key technologies in artificial seeds production of *P. ternata*.

Keywords

Pinellia ternata; Loose callus; Cell micromass; Artificial embryo; Suspension culture; Synchronization

Abbreviations:

2,4-D: 2,4-Dichlorophenoxyacetic Acid; 6-BA: 6-Benzylaminopurine; CH: Casein Hydrolysate; IBA: Indole-3-Butyric Acid; MS: Murashige and Skoog Medium; NAA: α-Naphthaleneacetic Acid

Introduction

Pinellia ternata (Thunb.) Breit. is a kind of medicinal plants (perennial herb) belonging to araceae, the medicinal parts are tubers which is an important major traditional Chinese medicine. It has many pharmacological effects such as dryness phlegm, downbear counterflow and check vomiting [1-3]. In recent years, the researchers found that *P. ternata* had the functions of antifertility, anticancer, antilipidemic and treating coronary heart disease [4-7]. According to statistics, there contain *P. ternata* in more than 200 kinds of proprietary Chinese medicine [8]. Because of the market demand increasing, together with the predatory excavation and changes of environment conditions, the medicinal materials of wild *P. ternata* appeared serious shortage. So artificial planting has become a major means to resolve the medicinal materials shortage of *P. ternata*. However, artificial planting easily led to lots of problems, such as the frequent damage of diseases [9-11], germplasm degeneration and yield decline [12-17] and it requires a large amount of reproduction materials and high cost. Although it provided a new approach that the tissue culture technology of *P. ternate* got success for to solve its reproduction and germplasm innovation, but the workload to transplant test-tube plantlets is very large, the survival rate is lower, and its management process is also complicated. Therefore, the technology has a slight practical significance in large-scale cultivation of *P. ternata*.

The emergence of artificial seed technology had brought expectation for the large-scale production of *P. ternata*, germplasm purification and rejuvenation [12,13,18-26]. The core of the technology is the synchronization culture of artificial embryos; it is also a key link relating to the mass production of artificial seeds can be fulfilled. So far, unfortunately, the technical issues of artificial embryos synchronization of *P. ternata* have failed to solve [18,20,26-34]. The reasons are that the intercellular junctions of *P. ternata* callus are very close and callus cells are difficult to disperse, so that the cells differentiation of callus different parts cannot be synchronized, and the small tubers (artificial embryos) by differentiation have no uniform size and they all connect tightly together, the operators have to adopt manual cutting to obtain independent small tubers using for artificial seeds embedding, it has seriously limited the artificial seeds industrialization of *P. ternata*. Therefore, it is a key and must first be solved technical problem in establishing artificial seeds industrialization system of *P. ternata* which how to culture the synchronization artificial embryos of dispersed to each other and suitable for artificial seeds production.

Our research team based on many years of trials [12,26,35], using the leaf blades or petioles as explants from aseptic seedlings of *P. ternata* to induce callus, then changing the intercellular bonding degree of callus cells by regulating carbon sources and plant growth regulators concentration and culture conditions to obtain the discrete and loose callus. Follow on to carry through the suspension culture to disperse callus cells into homogeneous cell masses (in this paper called "cell micromasses") suspension system which is the important basis of subsequent synchronization differentiation. Finally, we successfully constructed and optimized the technology system of artificial embryos synchronization culture of *P. ternata*. This study laid a reliable technological foundation for solving required a large number of excellent-quality seed stocks and seedlings in large-scale cultivation of *P. ternata*.

***Corresponding author:** Ming-Sheng Zhang, School of Life Sciences, Key Laboratory of Plant Resources Conservation and Germplasm Innovation in Mountainous Region (Ministry of Education), Guizhou University, 14, Xia-hui Rd., 550025, Guiyang, China, E-mail: mshzhang@163.com

Received: October 01, 2018 **Accepted:** October 16, 2018 **Published:** October 23, 2018

Materials and Methods

Experiment materials

Pinellia ternata (Thunb.) Breit. was provided by Bijie Agricultural Sciences Institute of Guizhou in China.

Culture conditions

In this experiment, the pH value of all media was 5.8, temperature was (25 ± 1)°C, illumination intensity was 1500 lx to 2000 lx; illumination time was 12 h/d. For particular conditions were specified otherwise.

Explants preparation

The tubers of *P. ternata* were washed with running water for 2 h, then to sterilize the tubers for 45 s by 75% alcohol and to wash 3 to 5 times by sterile water, and to sterilize for 8 min with mercury bichloride and to wash 3 to 5 times by sterile water. Finally, the tubers using sterile filter paper to dry surface water were inoculated on the culture medium (MS+1.0 mg/L 6-BA+0.1 mg/L IBA+30 g/L sucrose+6 g/L agar) to induce the asepsis seedlings. Selecting the vigorous seedlings of 15 d age, cutting the leaf blade into 0.5 cm^2 small pieces or 0.5 cm petiole short segments to use as the explants.

Callus induction and propagation

The orthogonal table of $L_9(3^4)$ was selected for studying the effects of three factors including 2,4-D, 6-BA and explant sources in callus induction (Table 1). Each treatment contained 5 test bottles, each bottle inoculated into 3 explants, to repeat 3 times, and regularly checking the induction rate and biomass (fresh weight) of the callus, so as to screen the appropriate media to suitable for callus induction and proliferation. At the same time, the fresh and well glossy callus selected were inoculated on the appropriate media to subculture and proliferate for obtaining discrete and loose callus which will be used for suspension culture.

Establishment and optimization of suspension culture system of cell micromasses

Selecting the discrete and loose callus to inoculate into the suitable liquid medium (above appropriate medium for callus proliferation, but not contain agar) for suspension culture (rotating speed 90 rpm). After 7 to 10 days, the suspension cultures were filtered through 40 mesh screen to obtain homogenous cultures. The homogenous cultures was continued to culture in MS medium containing different concentrations of 2,4-D, 6-BA, sucrose and casein hydrolysate (CH) to set up suspension system composed of uniform cell micromasses (synchronization). The suspension system was optimized through $L_9(3^4)$ orthogonal experiment which factors and levels were shown in Table 2. The medium bottling volume was 30 mL per bottle, the inoculation quantity was 2 g loose callus per bottle, inoculating 5 bottles per treatment, and repeated 3 times. The rotating speed of shaking table was 90 rpm. After 25 days, sucking cell micromass to use for subculture and proliferation. Meanwhile, filtering suspension cells to weigh and calculate the biomass.

Synchronization differentiation of cell micromasses and formation of artificial embryos

Sucking the uniform cell masses (synchronization cell masses) from continuous subculture 3 weeks to inoculate in basal medium (MS+10 g/L sucrose+300 mg/L CH) and adding different concentrations of 6-BA and IBA (Table 3). The medium bottling volume was 30 mL per bottle, the inoculation quantity was 50 cell micromasses per bottle, inoculating 5 bottles per treatment, and repeated 3 times, the illumination intensity was 3000 lx to 4000 lx, the rotating speed of shaking table was 50 rpm. The differentiation rate of cell micromasses and the amount of artificial embryos were calculated when the process of artificial embryos formation was basic completed.

Statistical methods

Callus induction rate=(Explant numbers of formed callus/Explant numbers of inoculation) × 100%.

Differentiation rate of cell micromasses=(Amount of artificial embryos/Inoculated amount of cell micromasses) × 100%.

The SPSS 20.0 and DPS v 7.05 software were used for statistical analysis in this test.

Results

Effects of explants and plant growth regulators for callus induction

The effects of callus induction rate and its biomass (fresh weight) by orthogonal combining experiment both explant based sites and plant growth regulators were analyzed. The results showed that the combination both explant parts and plant growth regulators had different degree influence for the biomass, color and texture of callus, but the difference was not too big for the induction rate of callus (Figure 1 and Table 1).

Table 1: Effects of different treatment combination for callus induction.

Treatments	A	B	C	Callus induction rate (%)*	Callus fresh weight (g)**	Color and textures of callus
	Explants	2,4-D (mg/L)	6-BA (mg/L)			
1	Leaf blade 0.5 cm × 0.5 cm	1	0.5	100	0.68 ± 0.02^{B}	Gray, relatively loose
2		1.5	1	100	0.72 ± 0.04^{B}	Yellow, relatively loose
3		2	1.5	100	0.93 ± 0.16^{A}	Faint yellow, loose
4	Petiole base 0.5 cm	1	1	95.6	0.49 ± 0.08^{BC}	Palegreen, dense
5		1.5	1.5	98.3	0.74 ± 0.09^{B}	Faint yellow, relatively loose
6		2	0.5	96.5	0.43 ± 0.04^{BC}	Gray, loose
7	Petiole top 0.5 cm	1	1.5	94.6	0.30 ± 0.01^{C}	Yellow, relatively dense
8		1.5	0.5	63.4	0.19 ± 0.05^{C}	Gray, relatively loose
9		2	1	93.8	0.26 ± 0.05^{C}	Faint yellow, loose

* Callus induction rate is mean of three replications. ** Callus fresh weight is mean ± standard deviation. *** Different capital letters at the end of figure on the same column indicate significant differences (P<0.01). Basal medium in this table is MS+sucrose 30 g/L+agar 6 g/L.

Table 2: Effects of different treatments combination for suspension culture of cell micromasses.

Treatments	A	B	C	D	Fresh weight of cell micromasses (g)
	2,4-D (mg/L)	6-BA (mg/L)	Sucrose (g/L)	CH (mg/L)	
1	0.5	0.2	30	100	8.10 ± 0.70^{b*}
2	0.5	0.5	40	200	8.87 ± 0.73^{ab}
3	0.5	1	50	300	7.27 ± 0.71^{b}
4	1	0.2	40	300	10.87 ± 0.27^{a}
5	1	0.5	50	100	9.50 ± 0.88^{ab}
6	1	1	30	200	8.53 ± 0.34^{ab}
7	1.5	0.2	50	200	9.70 ± 0.85^{ab}
8	1.5	0.5	30	300	9.73 ± 1.09^{ab}
9	1.5	1	40	100	7.53 ± 0.03^{b}
K_1	8.08	9.56	8.79	8.38	
K_2	9.63	9.57	9.09	9.03	
K_3	8.99	7.78	8.82	9.29	
R	1.56	1.78	0.30	0.91	

* Different lowercase letters at the end of figure on the same column indicate significant differences (P<0.05). The same below.

Table 3: Effects both 6-BA and IBA for cell micromasses to differentiate and form artificial embryos.

Treatments	Basal medium	Plant growth regulators		Amount of artificial embryos		
		6-BA (mg/L)	IBA (mg/L)	0.1 cm*	0.3 cm	≥ 0.5 cm
1	MS + sucrose 10 g/L + CH 300 mg/L	0	0	21.33 ± 2.31^{ab}	54.00 ± 1.73^{ab}	5.00 ± 2.65^{a}
2		0.5	0.05	25.33 ± 4.73^{a}	68.33 ± 6.43^{a}	0.67 ± 1.15^{b}
3		0.5	0.1	18.00 ± 4.58^{b}	49.00 ± 1.73^{c}	0.33 ± 0.58^{b}
4		0.5	0.2	11.67 ± 4.04^{c}	19.00 ± 8.66^{bc}	3.00 ± 2.00^{ab}
5		1	0.1	2.67 ± 2.52^{d}	20.00 ± 2.64^{d}	3.00 ± 0^{ab}
6		2	0.1	2.00 ± 1.73^{d}	43.00 ± 19.26^{d}	2.67 ± 2.52^{ab}

* 0.1 cm, 0.3 cm and 0.5 cm indicate the diameter of artificial embryos, respectively.

The callus appeared early after inoculating 7 to 10 days when the petiole base of *P. ternata* was used explants. At the 15th day, most of the treatments formed callus. The rate of callus formation reached the maximum at the 25th day. The high concentration of 6-BA was advantageous to the callus induction and proliferation, and the callus was more loose while adding high concentrations of 2,4-D into the medium. Overall, for callus induction and proliferation, leaf blade was the most explants, petiole base was second, and petiole top was less.

Comprehensive considerating the fresh weight and texture of callus, we thought that the combination of treatment 3 or treatment 5 was the suitable medium for callus induction of *P. ternata*. ie. the medium of suitable to leaf blade callus induction was MS+2.0 mg/L 2,4-D+1.5 mg/L 6-BA+30 g/L sucrose+6 g/L agar, and the medium of suitable to petiole callus induction was MS+1.5 mg/L 2,4-D+1.5 mg/L 6-BA+30 g/L sucrose+6 g/L agar. The callus was subcultured and proliferated on this medium, to get the discrete and loose callus which well be used for suspension culture to establish cell micromasses suspension system.

Culture conditions of suitable for cell micromasses formation and proliferation

The above callus was subcultured and proliferated for 3 times to form the discrete and loose callus (Figure 2A). The discrete and loose callus was evenly dispersed into suspension liquid after 7 to 10 days by suspension medium MS+2.0 mg/L 2,4-D+1.5 mg/L 6-BA+30 g/L sucrose (Figure 2B). At the moment, the suspension liquid contained some large callus chunk, the cells showed kidney-shaped (Figure 2C). Filtering out large callus chunk to obtain uniform and milky white cell micromasses. The cell micromasses were subcultured 3 times to form the suspension system of homogeneous cell micromasses. In the suspension system, the cells became spherical shape and proliferated rapidly (Figure 2D).

On the basis of preliminary experiment, screening medium compositions which had positive effect for suspension culture of cell micromasses carried out orthogonal combination experiment, the results were shown in Table 2 (basic culture medium was MS). By examining the fresh weight of cell micromasses, to find the fresh weight of cell micromasses in treatment 4 was the largest. Through extreme analysis, the contribution sorting of principal component for cell suspension culture was obtained, namely 6-BA＞2,4-D＞CH＞sucrose. The optimal combination of factors and levels as the medium for cell suspension culture was $A_2B_2C_2D_3$, in other words, the medium to suit for proliferation and growth of *P. ternata* cell micromasses was MS+1.0 mg/L 2,4-D+0.5 mg/L 6-BA+40 g/L sucrose+300 mg/L CH. The formation of homogeneous cell micromasses laid a solid foundation for to realize artificial embryos synchronization in artificial seeds production of *P. ternata*.

The effects of plant growth regulators ratio for cell micromasses synchronization differentiation to form artificial embryos

The homogeneous cell micromasses of *P. ternata* were continuous subcultured for 3 weeks by MS+1.0 mg/L 2,4-D+0.5 mg/L 6-BA+40 g/L sucrose+300 mg/L CH to form the larger cell masses (Figure 3A). Through the preliminary experiment based on different plant growth regulators combination, we found 6-BA and IBA combination showed a good effect for the cell micromasses synchronization differentiation to form artificial embryos. So 6-BA

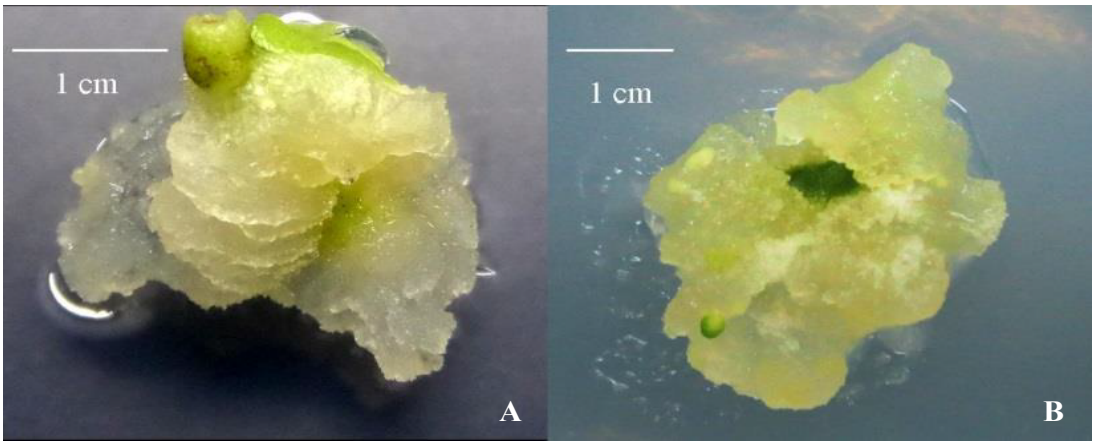

Figure 1: The callus from different explants of *Pinellia ternate* (**A)** Callus from petiole (**B)** Callus from leaf blade.

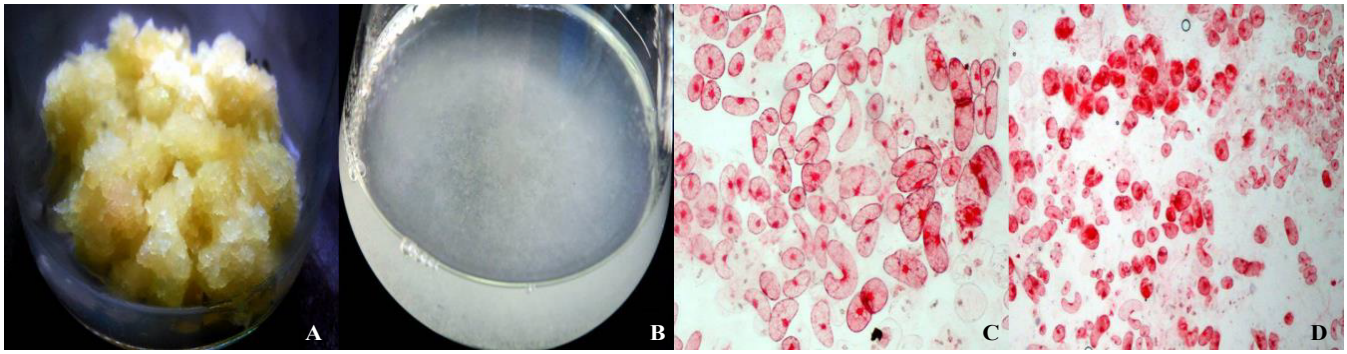

Figure 2: (**A)** Loose callus and cell micromasses (**B)** Discrete and loose callus (solid culture) (**C)** Cell micromasses (suspension culture) and (**D)** Cell micromasses under a microscope by aceto-carmine dyeing (200X).

and IBA combination were selected in the suitable ratio test which the larger cell masses by successive transfer culture for three weeks were used for synchronization differentiation to form artificial embryos, the results were shown in Figure 3, Figure 4 and Table 3.

The process of cell micromasses synchronization differentiation was experienced several stages (A, B, C and D in Figure 3). The different concentration combinations of 6-BA and IBA showed much difference for the effect both cell micromasses synchronization differentiation and artificial embryos formation, the combination of 0.5 mg/L 6-BA with 0.05 mg/L IBA was well (Figure 4 and Table 3). From Figure 4, we know that the differentiation rate of cell micromasses was increased with time extension of cultivation, the different treatments almost always took the 9th day as the inflection point. All cell micromasses were differentiated to form artificial embryos after 15 day. From Table 3, we can determine that the suitable medium for cell micromasses synchronization differentiation to form artificial embryos is MS+0.5 mg/L 6-BA+0.05 mg/L IBA+10 g/L sucrose+300 mg/L CH, and no matter in which a group of medium, its artificial embryos after differentiation were all about 0.3 cm in diameter as dominant. The results show that the synchronization degree was high from cell micromasses differentiation of *P. ternata* to artificial embryos formation.

Discussion

The yield and germination rate of *P. ternata* natural seeds are all lower, and its medicinal materials yield is lower and the production cycle is longer when the natural seeds are used as breeding materials. So, people mainly adopt the tubers propagation in artificial planting of *P. ternata*. Because *P. ternata* is a weak herbaceous plant and the ability to compete against weeds is weaker. In order to reduce the heavy weeding workload in artificial planting, the planting pattern of high density planting to suppress weeds was widely used [12,36]. Therefore, the dosage of seed stock is larger and its production cost is higher in the present production conditions *P. ternata*. In view of this phenomenon, it has become the key problem to realize the industrialization of medicinal materials that how to construct the scale production technology of *P. ternata* seeds or seedlings.

In recent years, a number of scholars from home and abroad have carried out the research on the seedlings breeding of *P. ternata*, but most concentrate on the test tube seedlings through tissue culture to induce callus [37-45] and because of the culture process cumbersome of test tube seedlings, transplant workload larger, survival rate low, production cycle long of the medicinal material, and so on, so farmers are hard to accept the test tube seedlings breeding. In other words, the technique by tissue culture to produce seedlings of *P. ternata* has little practical significance.

For important medicinal plants like *P. ternata* which natural seeds do not suitable for medicinal materials production, tubers propagation need very high cost and test tube seedlings are inconvenient to field operation, its artificial seeds production is undoubtedly a promising

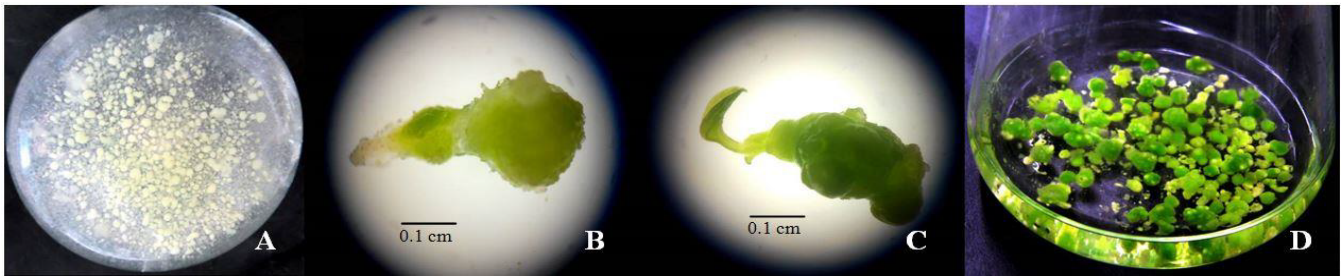

Figure 3: **(A)** The formation process on artificial embryos synchronization of *Pinellia ternate* (**B)** Cell masses by cell micromasses subculture (**C)** Artificial embryo of differentiating and developing (under the stereoscope) **(D)** Artificial embryos of completed differentiation and development.

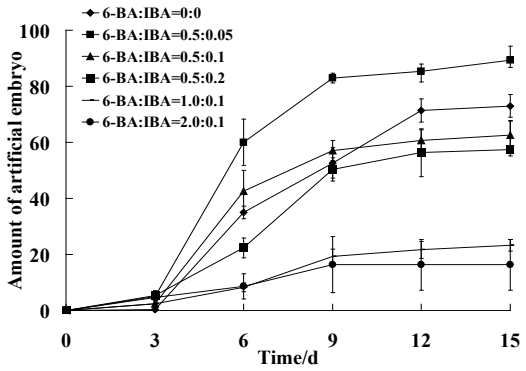

Figure 4: Differentiation rate of cell micromasses by different treatments.

technology choice, and this technology has also attracted many researchers. However, because artificial embryos of *P. ternata* must are the small tubers by callus differentiation and development, but the callus of *P. ternata* is quite tight and difficult to be dispersed, this is a fatal restriction factor for the embryo synchronization and it has not been broken through, causing the researchers almost still stay on these links such as artificial seed production process, artificial endosperm preparation and artificial seed coat screening, etc [13,20,39,46-48].

Conclusion

This study started with regulating carbon source and plant growth regulators concentration, cooperating with appropriate culture conditions, and solved effectively the problem which can reduce intercellular adhesion to form discrete and loose callus. Based on this, we established the dispersive and homogeneous cell micromasses suspension system; it realized the first breakthrough of *P. ternata* artificial embryos synchronization technology. This research work laid a good foundation for to build the industrialization technology of *P. ternata* artificial seeds.

Conflict of Interest

This research belongs to the research project of the tutor, which is accomplished by the unified guidance of the tutor in the same laboratory. There is no conflict of interest. All the data and materials in this paper are reliable and available from our lab.

Acknowledgements

This research was funded to MSZ by a grant from the National Key Research and Development Project in China (No. 2016YFC050260403), the Major Special Project of Science and Technology Plan in Guizhou of China (No. 2017-5411-06), the Science and Technology Support Plan Project in Guizhou of China. (No. 2018-2797), the Special Fund of Science and Technology Innovation Talent Team Construction in Guizhou of China (No. 2016-5624), the Project of High-level Innovative Talents in Guizhou of China (No. 2015-4031), the Major Projects of Innovation Group in Guizhou of China (No. 2016-023), the National key cultivation project of Guizhou University (No. 2017-5788), and the Modern Industrial Technical System Construction Project of Chinese Medicinal Materials in Guizhou of China (No. GZCYTX-02).

References

1. Wang ZQ, Li BC (2009) Research progress on pharmacological activities of *Pinellia ternate*. Shanxi Med J 38: 65.
2. Chinese Pharmacopoeia Commission (2015) China Pharmacopeia (Volume I). China Medical Science and Technology Press, Beijing.
3. Yu HL, Zhao TF, Wu H, Pan YZ, Zhang Q, et al. (2015) *Pinellia ternata* lectin exerts a pro-inflammatory effect on macrophages by inducing the release of pro-inflammatory cytokines, the activation of the nuclear factor-κB signaling pathway and the overproduction of reactive oxygen species. Int J Mol Med 36: 1127-1135.
4. Zhang YL, Li GR, Wei YL (2007) Advances in research of traditional Chinese Medicine *Pinellia ternata* (Thunb) Berit. Chin Agric Sci Bull 23: 163-167.
5. Li XG, Lu P, Zhang WF, Li B, Yang R, et al. (2013) Study on anti-Ehrlich ascites tumour effect of *Pinellia ternata* polysaccharide *in vivo*. Afr J Trad Complem Altern Med 10: 380-385.
6. Ji X, Huang Bk, Wang GW , Zhang CY (2014) The ethnobotanical, phytochemical and pharmacological profile of the genus *Pinellia*. Fitoterapia 93: 1-17.
7. Zhang XT, Cai YJ, Wang LJ, Liu HC, Wang XL (2015) Optimization of processing technology of Rhizoma *Pinellia* Praeparatum and its anti-tumor effect. Afr Health Sci 15: 101-106.
8. Cheng XY, Yang ZQ (2013) Optimization study of callus induction on *Pinellia ternata*. Tianjin Agric Sci 19: 4-6.

9. Hu XF, Ying FX, He YB, Gao YY, Chen HM, et al. (2008) Characterization of Pectobacterium carotovorum subsp. carotovorum causing soft-rot disease on *Pinellia ternata* in China. Eur J Plant Pathol 120: 305-310.
10. Hu XF, Fang QL, Li SX, Wu JG, Chen JS (2009) Isolation and characterization of endophytic and rhizosphere bacterial antagonists of soft rot pathogen from *Pinellia ternata*. FEMS Microbiol Lett 295: 10-16.
11. Zeng LX, Li DY (2009) Investigation and integrated management for diseases and pests on *Pinellia ternata* in Guizhou. J Guizhou Agric Sci 37: 92-95.
12. Zhang MS, Li H (2009) Studies on preparation technique and germination of *Pinellia ternata* artificial seeds. Seed 28: 4-6.
13. He YK, Zhu CP, He MY, Hu AL, Hao S (1997) Morphogenesis of tubercles and production of artificial seeds in *Pinellia ternata*. Acta Agronomica Sinica 32: 42-49.
14. Zheng YM (2008) The effect factors on the yield of *Pinellia*. Mod Agric Sci 15: 17-18.
15. Chen L, Xu HY, Zhou FJ, Wei LJ, Tang LX, et al. (2008) Bio-effectives of different prescription with NAA and N^6-BA on the tissue culture of *Pinellia ternata* [Thunb.] Breit *in vitro*. Seed 27: 30-33.
16. Peng XQ, Zhang WH (2011) Technology system for stem-apex meristem culture and rapid progagation of *Pinellia ternata* (Thunb.) Breit. J Jinggangshan Univ 32: 42-49.
17. Tao ML, Liu QB, Huang HM (2013) Establishment of culture system of *Pinellia ternata* (Thunb.) Breit. Northern Hortic 4: 113-117.
18. Thorpe T (1978) Frontiers of plant tissue culture. Calgary University Press, Calgary, Canada.
19. Chen DF, Chen XW, Cheng BS (1995) Discussion on several problems artificial seed. J Shandong Agric Univ 26: 249-256.
20. Zhang SF (1998) Study on the artificial seeds of *Pinellia ternata*. J Xinyang Teachers Coll 11: 281-282.
21. Zhang M, Huang HR, Wei XY (2000) Adeances in study on artificial seed of plant. Chin Bull of Bot 17: 407-412.
22. Saiprasad GVS (2001) Aritificial seeds and their applications. Resonance 6: 39-43.
23. Choi YE, Jeong JH (2002) Dormancy induction of somatic embryo of Siberian ginseng by high sucrose concentrations enhances the conservation of hydrated artificial seeds and dehydration resistance. Plant Cell Rep 20: 1112-1119.
24. Xue JP, Zhang AM, Ge HL, Sheng W (2004) Technique on artificial seeds of *Pinellia ternata*. China J Chin Mat Med 29: 402-405.
25. Ding SH, Zhang MS, Shi MN (2011) Advances on the technology of artificial plant seeds. Seed 30: 60-66.
26. Shen QF, Zhang MS, Li LQ, Liu GX (2014) The effects of plant growth regulators on artificial embryo formation of *Pinellia ternata*. J Mount Agric Biol 33: 33-36.
27. Kamda H (1985) Practical tecnology on the mass production of clonal plant. Artificial Seed: 48.
28. Tsay HS, Gau TG, Chen CC (1989) Rapid clonal propagation of *Pinellia ternata* by tissue culture. Plant Cell Rep 8: 450-454.
29. Standard A, Piccioni E (1998) Recent perspectives on synthetic seed technology using nonembryogenic *in vitro*-derived explants. Int J Plant Sci 159: 968-978.
30. Zhan ZG, Zhang M, Xu C (2001) Plant non-embryogenesis and artificial seeds. Seed 6: 28-30.
31. Borges M, Ceiro W, Meneses S, Aguilera Vazquez J, Infante Z, et al. (2004) Regeneration and multiplication of *Dioscorea alata* germplasm maintained *in vitro*. Plant Cell Tiss Organ Cult 76: 87-90.
32. Belarmino MM, Gonzales JR (2008) Somatic embryogenesis and plant regeneration in purple food yam (*Dioscorea alata* L.). Ann Trop Res 30: 22-33.
33. Rai MK, Jaiswal VS, Jaiswal U (2008) Alginate-encapsulation of nodal segment of guava (*Psidium guajava L*) for germplasm exchange and distribution. Hortic Sci Biotechnol 83: 569-573.
34. Munetaka Sugiyama (2015) Historical review of research on plant cell dedifferentiation. J Plant Res 128: 349-359.
35. Liu GX, Zhang MS, Li XL, Hu SS, Wei HB, et al. (2016) Induction and multiplication of loose callus of *Pinellia ternata* (Alismatales□Araceae) suitable for suspension culture. J Mount Agric Biol 35: 82-85.
36. Li H, Zhang MS, Peng SW, Xu L (2009) Economic benefits of different *Pinellia ternata* (Thunb.) Breit. growing modes. Mod Chin Med Mat Med 11: 566-569.
37. Shoyama Y, Hatano K, Nishioka I (1983) Clonal multiplication of *Pinellia ternata* by Tissue Culture. Planta Med 49: 14-l6.
38. Mao ZC, Peng ZS (2003) Progress on research of rapid propagation system of *Pinellia ternata*. China J Chin Mat Med 28: 193-195.
39. Xue JP, Zhu YF, A Zhang M, Liu J (2004) Research on direct formation of microtubers from *Pinellia ternata*. Acta Agronomica Sinica 30: 1060-1064.
40. Xu XM, Lv DX, Zhang M, Zhang M, Chen M (2005) Clusteres shoot induction and rapid propagation on *Pinellia ternata*. J Chin Med Mat 28: 1052-1053.
41. Peng ZS, Luo CK, Cai P, Mao ZC, Kang CY, et al. (2007) Rapid propagation of the medicinal plant *Pinellia ternata* by *in vitro* leaves culture. Bulg J Agric Sci 13: 1-6.
42. Li XL, Wang XM (2008) Study on the rapid-propagation technical system with the petioles of *Pinellia ternata* Breit as explants. J Anhui Agric Sci 36: 2247-2248.
43. Wang JL, Wang Q, Wang J, Lu Y, Xiao X, et al. (2009) Effect of different plant growth regulators on micro-tuber induction and plant regeneration of *Pinellia ternata*(Thunb) Briet. Physiol Mol Biol Plant 15: 359-365.
44. Song YM, Li F, Liu Y (2010) Study on tissue culture and rapid propagation of *Pinellia ternata*. J Shandong Univ TCM 34: 368-369.
45. Liu XX, Cui XX, Liu JX, Meng FY, Wei YQ (2011) Different plant growth regulators on callus induction of *Pinellia ternata*. Chin J Inform TCM 18: 57-59.
46. Kim SW, Dong SI, Tae KH, Liu JR (2005) Somatic embryogenesis and plant regeneration in leaf and petiole explant cultures and cell suspension cultures of *Pinellia tripartite*. Plant Cell Tiss Organ Cult 80: 267-270.
47. Xue JP, Zhang AM, Sheng W, Gao X (2005) Storage technique on artificial seeds of *Pinellia ternata*. China J Chin Mat Med 30: 1820-1823.
48. Wang HX, Cai ZP, Qi YL, Wei LX, Li YP (2011) Effects of different embedding matrix on germination rate artificial seed of *Pinellia* . China Seed Ind 6: 59-60.

Author Affiliations

Top

School of Life Sciences, Key Laboratory of Plant Resources Conservation and Germplasm Innovation in Mountainous Region (Ministry of Education), Guizhou University, Guiyang, 550025 Guizhou, People's Republic of China