Research Article

Effect of different plant growth regulators on micro-tuber induction and plant regeneration of *Pinellia ternate* (Thunb) Briet

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ABSTRACT

An efficient micropropagation system for *Pinellia ternate* (Thunb) Briet, a traditional Chinese medicinal plant, has been developed. Petiole and lamina of *P. ternate* were used as explants and cultured on Murashige and Skoog (MS) medium containing different concentrations of different plant growth regulators. The results indicated that low concentration of 2,4-dicholorophenoxy acetic acid (2,4-D), indole-3-acetic acid (IAA) and α-naphthalene acetic acid (NAA) were suitable for micro-tuber induction, but callus induction rate increased with increasing concentrations of growth regulators. Tubers induction rates of petiole and leaf were (81.8 % - 100 %) and (89.4 % - 96.0 %) respectively, when 0.2 mg l^{-1} 2, 4-dicholorophenoxy acetic acid, indole-3-acetic acid or α-naphthalene acetic acid were present in the medium. Tubers induction rates of petiole and leaf cultured on MS medium supplemented with 0.2 - 0.5 mg l^{-1} 6-benzyl amino purine (6-BAP) were (94.1 % - 100 %) and (96.0 % - 100 %) respectively. When the concentration of 2,4-dicholorophenoxy acetic acid, α-naphthalene acetic acid and 6-benzyl amino purine was increased to 2.0 mg l^{-1}, callus induction rates of petiole and leaf were 100 % and 98.2 %, 91.0 % and 36.0 %, 62.3 % and 70.0 %, respectively. Different concentration of kinetin (KT) and zeatin (ZT) had no significant effect on micro-tuber induction of petiole. Most petioles showed polarity during the cultivation of explants, when supplemented with different concentrations of auxin or cytokinin in the MS medium. **[Physiol. Mol. Biol. Plants 2009; 15(4) : 359-365]** *E-mail : wangjunli2008@yahoo.com.cn*

Keywords : *Pinellia ternate (Thunb) Briet; Plant growth regulator; Micro-tuber; Regeneration*

INTRODUCTION

Pinellia ternate (Thunb) Briet is a perennial herb belonging to the Araceae family. There are eight species in the world, seven widely distributed domestic species, of which six species are unique to China (Zhang *et al.,* 2006). The tuber of *P. ternate* is one of the main components in many traditional Chinese medicines. According to an ancient Chinese book on medicine, *Shen Nong Ben Cao Jing,* the properties of *P. ternate* are described as pungent, warm in nature, and toxic. It has pharmacological functions like that of reducing lipid, expectorant, anti-spasm, detumescence, anti-emetic (relieves vomiting), anti-tumor and anti-fertility, and treatment of coronary heart disease (Drugs institute of Chinese Academy of Medical Sciences, 1993). *P. ternate* can also be used as antimicrobial agent (Chen *et al.*, 2003). Naturally, it has garnered much attention locally as well as globally; with Japan and Southeast Asian countries importing this herbal medicine mainly from China (Luo and Peng, 2005).

Phytochemical study of the rhizome of *P. ternate* by RP-LC and isolation of four phenylpropanoids -(E)-p-coumaryl alcohol, 3,4-dihydroxycinnamyl alcohol, sachaliside 1, and coniferin from the rhizome, have been reported (Han *et al.,* 2006). The *Pinellia ternate* agglutinin (PTA), a monocot mannose-binding lectin that catalytically agglutinated rabbit erythrocytes, was also isolated from the tubers of *Pinellia ternate*. The potential effect of PTA has gained considerable interest in recent years owing to the clinical use of native PTA against

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cancer and for protecting plants from insect pests. A successful strategy to allow high-level expression of PTA as inclusion bodies in *Escherichia coli* M15 has been reported (Lin *et al.,* 2003). *Pinellia ternate* agglutinin gene was transformed into wheat to render increased resistance to oriental armyworm and aphid (Yu and Wei, 2008).

In the recent years, the wild resource of *P. ternate* is at the brink of exhaustion because of excessive usage (Mao and Peng, 2003). *P. ternate* can be propagated through tubers, seed-breeding and buds, but the coefficient of reproduction is lower. Micropropagation techniques play an important role in propagule production for cultivation and conservation of different medicinal plant species such as *Eucommia ulmoides* (Wang *et al.,* 2003), *Salvia africana-lutea* L. (Makunga and van Staden, 2008), *Catharanthus roseus* (Dhandapani *et al.,* 2008).

In vitro culture of *P. ternate* has been reported (Shoyama *et al.,* 1983; Ren *et al.,* 1983; Zeng *et al.,* 1995; Luo *et al.,* 2000; Mao and Peng.2003; Xue *et al.,* 2004; Fan *et al.,* 2005; Chang *et al.,* 2007; Peng *et al.,* 2007) where petiole or leaves were used as explants, and tubers were induced directly. However, the induction and reproduction process at various combinations of regulators, as well as other aspects of culture has not yet been studied thoroughly. In this paper, a successful and efficient protocol for the induction of tuber from petiole and leaf of *P. ternate* was developed, and the responses of explants to various plant growth regulators were also discussed.

MATERIALS AND METHODS

Plant material and preparation of sterile plantlets

Stem tubers of *P. ternate* collected from Xihe, Gansu province of China, were used as initiation material in this study. The tubers were thoroughly washed with tap water, and surface sterilized with 75 % alcohol for 30 s and 0.1 % (w/v) mercuric chloride solution ($HgCl_2$) for 20 min and then rinsed 3 times with sterile deionized water. They were inoculated vertically in 100 ml triangle glasses (3-5 tubers per glass) with 30 ml of MS medium (Murashige and Skoog, 1962), supplemented with IAA, NAA or 2,4-D (1.0 mg l^{-1}), sucrose 30 g l^{-1}, and agar 7 g l^{-1}. The pH of the medium was adjusted with 1 M NaOH to 5.8 before being autoclaved at 121 ºC for 20 min. and the sterilized tubers were cultured under illumination (30-40 µmol m^{-2} s^{-1}) at 25-26 ºC.

Micro-tuber induction

Petiole (0.5-1.0 cm) and lamina (0.5 cm×0.5 cm) cutting from sterile plantlets of *P. ternate* were used as explants and cultured on MS medium. Testing of the effect of various concentrations of different plant growth regulators on micro-tuber induction was performed on the MS medium supplemented with IAA, NAA, 2,4-D, 6-BAP, KT and ZT at various concentrations (0.2, 0.5, 1.0, 2.0 mg l^{-1}), alone, enriched by sucrose 30 g l^{-1} and agar 7 g l^{-1}. In all cases, the pH of the medium was adjusted with 1 M NaOH to 5.8 before being autoclaved at 121 ºC for 20 min.

Petri dishes (10 cm in diameter) were used to culture the petiole and lamina explants, each containing 30 ml of culture medium with 10 explants inoculated per dish. All cultures were incubated under 30-40 µmol m^{-2} s^{-1} light provided by a white, cool fluorescent tube at a photoperiod of 12 h at 25-26 ºC. After 4 weeks of culture, callus induction rate and micro-tuber induction rate was calculated.

Acclimatization

Regenerated plantlets were washed carefully in running tap water to remove the traces of agar and transferred to pots containing peat moss and vermiculite (2:1). The plantlets were initially irrigated with quarter-strength inorganic salts of MS medium for 2 weeks followed by tap water. Potted plantlets were grown in room conditions (25-26 °C) for 2 weeks and acclimatized plantlets were transferred to the greenhouse.

Data analysis

LSD values were calculated at a 5 % significance level. Micro-tuber induction rate (number of the explants forming tubers / total number of explants cultured × 100) and callus induction rate (number of the explants forming callus /total number of explants cultured × 100) were calculated for the explants that had been cultured for 4 weeks.

RESULTS AND DISCUSSION

The effect of different plant growth regulators on germination of stem tubers

Stem tubers of *P. ternate* germinated in one week on the medium supplemented with growth regulators and shoot proliferation took place four weeks later. When IAA 1.0 mg l^{-1} was present in MS medium, tubers germinated and grew rapidly without any significant

differences in morphology, as compared to the tubers growing on regulator-free medium. When NAA 1.0 mg l^{-1} was added into MS medium, roots induced radially and changed into coarse and green. On MS medium with 1.0 mg l^{-1} 2, 4-D induced roots became rough and short, with a large number of villi and a little callus. In order to get higher plantlets induction, medium supplemented with IAA and NAA was much better than medium with 2,4-D.

The induction effect of 2,4-D

Different concentrations of 2,4-D showed different induction effects on explants. Lower concentration of 2,4-D was in favour of induction of micro-tubers. When concentration of 2,4-D was as high as 0.2 mg l^{-1}, micro-tuber induction rates of petiole and leaf were 100 % and 89.4 % respectively. Induction rates of petiole and leaf decreased to 80.6 % and 77.8 % when 0.5 mg l^{-1} 2,4-D was supplemented in the medium. Many green micro-tubers were induced on the surface of explants and most of the explants were completely covered with small tubers. High concentration of 2,4-D was conducive to callus induction. When the concentration of 2,4-D increased to 1.0 mg l^{-1}, the pale yellow callus could be induced at the rates of 93.3 % and 63.6 % for petiole and leaf, respectively. When 2,4-D concentration increased to 2.0 mg l^{-1}, the callus formed rough and short roots with villi and the callus induction rates of petiole and leaf increased to 100 % and 98.2 % (see table 1 and figure 1 A-C).

The induction effects of IAA

Low concentration of IAA was efficient for the induction of micro-tubers. When the concentration of IAA was as high as 0.2 mg l^{-1}, micro-tuber induction rates of petiole and leaf were 81.8 % and 92.0 %, respectively. With the concentration of IAA increasing to 2.0 mg l^{-1}, micro-tuber induction rates of leaf decreased sharply to 22.5 %, while callus induction rates of petiole and leaf were 40.7 % and 2.5 % and many roots were induced on the callus. The results showed that high concentration of IAA was more conducive to callus induction for petiole explants, inhibiting differentiation of tubers for leaf explants (see table 1 and figure 1 D-E).

The induction effects of NAA

NAA can promote the formation of more tubers and roots (see figure 1F). When the concentration of NAA was at 0.2 mg l^{-1}, micro-tuber induction rates of petiole and leaf were 94.5 % and 96.0 %, respectively. With the increasing concentration of NAA, the induction rates

Table 1. Effect of different plant growth regulators on the induction rates of the explants of *P. ternate*

Different plant growth regulator (mg/l)		Induction rates of tubers (%)		Callus induction rates (%)	
		petiole	leaf	petiole	leaf
2,4-D	0	0 a	0 a	0 a	0 a
	0.2	100 b	89.4 b	50.0 b	10.6 b
	0.5	80.6 b	77.8 b	93.5 c	86.7 c
	1.0	20.0 c	43.0 c	93.3 c	63.6 d
	2.0	5.0 ac	38.6 c	100 c	98.2 e
IAA	0	0 a	0 a	1.5 a	0 a
	0.2	81.8 b	92.0 b	13.6 b	0 a
	0.5	64.9 b	82.0 bc	21.6 c	8.0 b
	1.0	72.7 b	73.9 c	33.6 c	0 a
	2.0	71.3 b	22.5 d	40.7 c	2.5 a
NAA	0	2.1 a	0 a	1.5 a	0 a
	0.2	94.5 b	96.0 b	21.8 b	0a
	0.5	61.8 cd	61.4 c	56.4 c	0 a
	1.0	73.8 c	42.0 d	82.0 d	34.0 b
	2.0	52.3d	40.0 d	91.0 d	36.0 b
6-BA	0	0 a	0 a	0 a	0 a
	0.2	94.1 b	100 b	1.9 a	0 a
	0.5	100 b	96.0 b	0 a	2.0 a
	1.0	73.3 c	78.3 c	41.7 b	20.0 b
	2.0	58.2 c	60.0 d	62.3 c	70.0 c
KT	0	0 a	0 a	0 a	0 a
	0.2	80.7 b	35.3 b	5.8 ab	0 a
	0.5	82.4 b	54.0 b	17.6 bc	4.0 a
	1.0	73.8 b	75.0 c	21.7 c	17.5 c
	2.0	83.0 b	58.0 b	50.9 d	0 a
ZT	0	0 a	0 a	0 a	0 a
	0.2	73.8 b	78.4 b	11.9 b	0 a
	0.5	77.1 b	80.0 b	16.7 b	4.0 a
	1.0	86.7 b	86.0 b	25.0 b	10.0 b
	2.0	82.2 b	78.4 b	48.9 c	29.4 c

The results were calculated from two replicated experiments, each with 50 explants per treatment. The values with different letters are significantly different ($P<0.05$) using the LSD test.

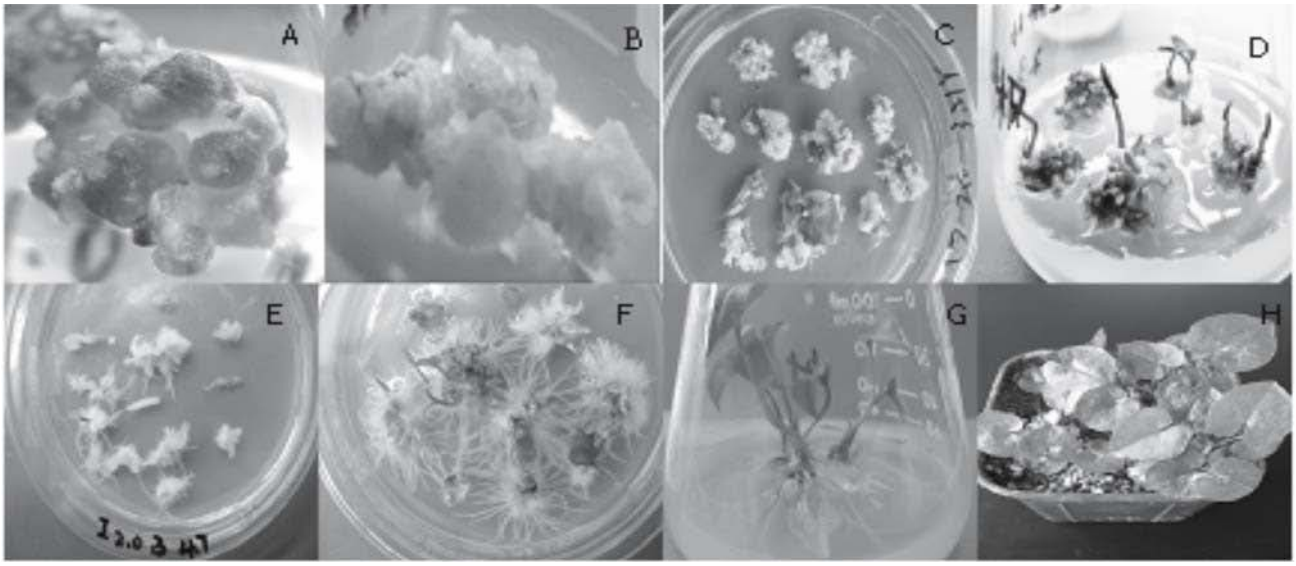

Fig. 1. The induction effects of different plant growth regulators and regenerated and transplanted plants of *P. ternate*. A: micro-tubers induced on medium with 0.2 mg l^{-1} 2,4-D, B: the pale yellow callus induced plus 1.0 mg l^{-1} 2,4-D, C: the callus formed rough and short roots with villi on medium with 2.0 mg l^{-1} 2,4-D, D: micro-tubers induced with 0.5 mg l^{-1} IAA, E: the callus and roots induced with 2.0 mg l^{-1} IAA, F: micro-tubers and roots induced with 0.2 mg l^{-1} NAA, G: regenerated plants in the triangle glass, H: transplanted plants in the small pot.

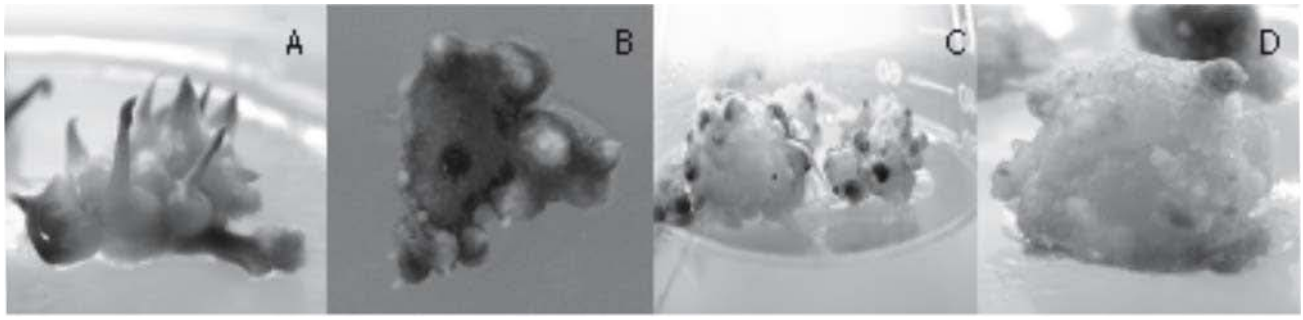

Fig. 2. Formation of micro-tubers from different explants. A: micro-tubers formed directly form petiole, B: micro-tubers formed directly form leaf, C and D: callus formed form petioles and leaves explants at first and then micro-tubers formed from the callus.

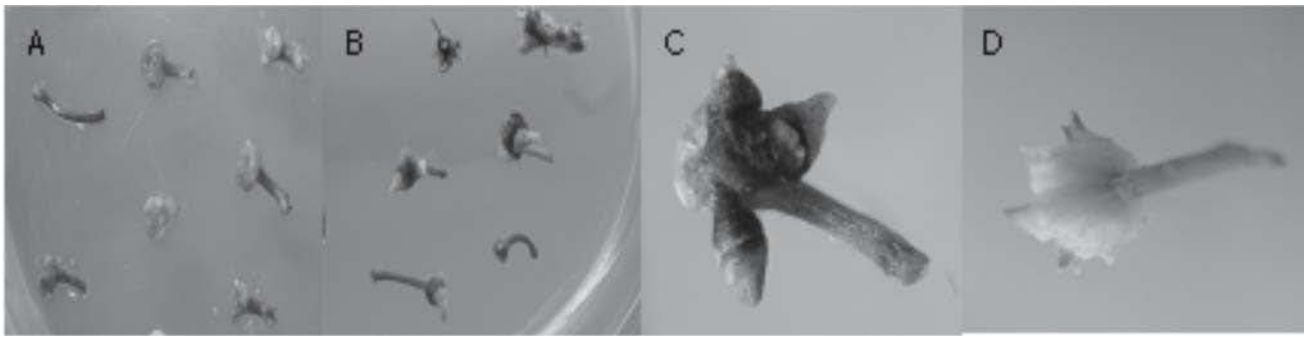

Fig. 3. Polarity of petioles. A: enlarged bottoms of petioles, B: micro-tubers formed from the bottoms of petioles, C: micro-tubers formed from the bottom of a single petiole, D: enlarged bottom of a single petiole.

of micro-tubers decreased gradually, while callus induction rate increased. When the concentration of NAA increased to 2.0 mg l^{-1}, micro-tuber induction rates of petiole and leaf decreased to 52.3 % and 40 %, while the callus induction rates of petiole and leaf were 91 % and 36 %, respectively (see table 1). These results showed that low concentration of NAA was suitable for differentiation of micro-tubers and roots, and high concentration of NAA was efficient for callus induction. This result was similar to the report of Fan *et al.* (2005).

Contrary to our results in *Dioscorea nipponica*, micro-tubers were also produced when the concentration of NAA was at 0.5–2.0 mg l^{-1} (Chen *et al.,* 2007). However, the percentage of micro-tubers increased with increasing of NAA from 0.5 to 2.0 mg l^{-1} and high concentrations of NAA promoted the formation of more and heavier micro-tubers of *D. nipponica,* which confirm the different responses of plant species to different plant growth regulators.

The induction effects of 6-BAP

Lower concentration of 6-BAP was conducive to the induction of micro-tubers. When concentration of 6-BAP was as high as 0.2-0.5 mg l^{-1}, micro-tuber induction rates of petiole and leaf were 94.1-100 % and 96.0-100 %. When the concentration of 6-BAP increased to 2.0 mg l^{-1}, micro-tuber induction rates of petiole and leaf decreased to 58.2 % and 60.0 %, while callus induction rates of petiole and leaf increased to 62.3 % and 70.0 %, respectively. Green callus formed from both ends of petioles and edges of leaves (see table 1).

To find out the high efficiency technical system for rapid propagation of *P. ternate*, the petioles were cultured on different media. The results from previous studies indicated that MS medium with 0.5 mg l^{-1} 6-BAP and 0.2 mg l^{-1} NAA was appropriate to micro-tuber induction and an increased number of tubers of better quality can be induced from the petioles (Li and Wang, 2008). This report indicated the combinatorial effect of 6-BAP and NAA but without any independent effect of 6-BAP or NAA.

The induction effects of KT

Micro-tuber induction rates of petiole and leaf were 73.8 % - 83.0 % and 35.3 % -75.0 % respectively with different concentrations of KT. Higher concentration of KT supported callus formation from the petioles. When the concentration of KT increased to 2.0 mg l^{-1}, callus induction rate of petiole was as high as 50.9 %, but the leaves did not form any callus (see table 1).

The induction effects of ZT

Micro-tuber induction rates of petiole and leaf were 73.8-86.7 % and 78.4-86.0 % respectively with different concentrations of ZT. With the increasing of concentration, callus induction rate increased. When the concentration of ZT increased to 2.0 mg l^{-1}, the callus induction rates of petiole and leaf increased to 48.9 % and 29.4 % respectively (see table 1).

Pathways to the formation of micro-tubers

The plantlets of *P. ternate* could regenerate from the lamina and petiole directly without the callus phase. According to Xu *et al.* (2005), the MS medium with 2.0 mg l^{-1} 6-BAP and 0.1 mg l^{-1} NAA was optimal for the induction of clustered shoots from explants of lamina, and the MS with 2.0 mg l^{-1} 6-BAP and 0.25 mg l^{-1} NAA was the best for the explants of petiole. Lee and Lee (2003) reported that the optimum growth regulator concentration for regeneration from totipotent callus of *Cypripedium formosanum* was found to be 4.44 mM 6-BAP, where the highest percentage of protocorm-like body (PLB) formation was 96.2 % and the maximum number of PLBs per explant was 13. The medium without growth regulators or containing 2, 4-D alone resulted in PLB formation, but with a lower number of PLBs. On this medium, most callus turned yellowish green and became compact in appearance under illumination that gave rise to a lower percentage of PLB formation (11.1-12.9%).

To study the effects of different factors on direct induction of micro-tubers, petiole and leaf explants of *P. ternate* were cultured in different MS medium using the orthogonal design method. The result showed that the optimal medium for direct induction of micro-tubers from leaves were MS with 0.5 mg l^{-1} 6-BAP and MS with 0.5 mg l^{-1} 6-BA and 0.5 mg l^{-1} IAA (Zhang *et al.,* 2005). Our results were similar to this report.

There were two ways to form micro-tubers in our test. One way is micro-tuber formation directly from petioles and leaves explants without callus. The other way is callus formation form petioles and leaves explants at first and then micro-tuber formation from the callus (see figure 2A-D).

Polarity of petiole

Most petioles showed polarity during the cultivation of explants, whether supplemented with different concentrations of auxin or cytokinin in the MS medium. Micro-tubers or callus formed only at the bottom when

petioles were inoculated horizontally or vertically (see figure 3A-D). This may be related to the different distribution of the hormones in the explants.

The formation of plant tubers is an extremely complex process, involving plant growth, development, metabolism, and a series of dynamic changes. IAA can promote the formation of tubers, and the increase of endogenous IAA is conducive to the formation of tubers. Jarvis *et al.* (1983) also confirmed that the increased concentration of IAA induced the increased synthesis of RNA, which translated specific proteins and induced the formation of tubers.

To explore the physiological and biochemical mechanism of tuber development, analysis of variation and function of five endogenous hormones (IAA, GA_3, ABA, ZR and JA) in the formation of micro-tubers of *P. ternate in vitro* has been studied by Chang *et al.,* (2007). The results showed that all the micro-tubers grew in the physiological upper ends when the petioles were inserted upside down. Enzyme linked immunosorbent assays showed that the content of endogenous hormones IAA, ABA, ZR and JA in the physiology of upper ends increased in the process of tuber formation, but GA_3 decreased sharply. With the formation of tubers, the balance of GA_3 and other hormones changed.

Acclimatization and transplantation of regenerated plantlets

As a result of our experiments, many micro-tubers induced and germinated into complete regenerated plantlets. Before the regenerated plantlets were transplanted into the field, the acclimatization of *in vitro* plantlets was necessary for adaptation to the environment. Regenerated plants were planted into small pots containing peat moss and vermiculite (2:1) for further growth in the solar greenhouse. After 30 d, more than 90 % plants survived (see figure1 G-H).

In conclusion, the independent effects of 2,4-D, IAA, NAA, 6-BAP, KT and ZT were studied in this paper, and the results showed that low concentrations of 2,4-D, IAA, NAA and 6-BAP were conducive to the formation of micro-tubers and did not promote the callus induction. High concentrations of growth regulators were efficient in callus induction but inhibited the formation of micro-tubers.

ACKNOWLEDGMENT

This work was financially supported by National Basic Research Program of China (973 Program, 2009CB522300), the Fund of State Key Laboratory of Phytochemistry and Plant Resources in West China , the “985” project (CUN985-3-3) and “211” project, P.R. China.

REFERENCES

Chang L, Xu YM and Xue JP (2007). Formation of microtubers from the petiole of *Pinellia ternate* (Thunb.) Berit. in vitro and change of endogenous hormones content (Chinese). *Journal of Huazhong Agricultural University,* 26(5): 612-615.

Chen FQ, Fu Y, Wang DL, Gao X and Wang L (2007). The effect of plant growth regulators and sucrose on the micropropagation and microtuberization of *Dioscorea nipponica* Makino. *Journal of Plant Growth Regulation,* 26: 38-45.

Chen JH, Cui GY, Liu JY and Tan RX (2003). Pinelloside, an antimicrobial cerebroside from *Pinellia ternate*. *Phytochemistry,* 64: 903-906.

Dhandapani M, Doo Hwan Kim and Seung Beom Hong (2008). Efficient plant regeneration via somatic embryogenesis and organogenesis from the explants of Catharanthus roseus. *In vitro Cells Dev. Biol.-Plant,* 44: 18-25.

Drugs Institute of Chinese Academy of Medical Sciences (1993). History of Chinese Medicine (book 2) (Chinese). People’s Health Press, Beijing 28-29.

Fan MH, Zhou JY, Peng Y and Liu SN (2005). Effect of induction and proliferation of callus of *Pinellia ternate* (Chinese). *Journal of Shandong Chinese Medicine,* 24(3): 168-171.

Han MH, Yang XW, Zhang M and Zhong GY (2006). Phytochemical study of the rhizome of *Pinellia ternate* and quantification of phenylpropanoids in commercial *Pinellia* tuber by RP-LC. *Chromatographia,* 64: 647-653.

Lin J, Yao JH, Zhou XW, Sun XF and Tang KX (2003). Expression and purification of a novel mannose-binding lectin from *Pinellia ternate*. *Molecular Biotechnology,* 25: 215-221.

Jarvis BC, Shannon PRM and Yasmin S (1983). Influence IBA and cordyeepin on rooting and RNA synthesis in stem cutting of *Phaseolus arureus* Roxb. *Plant Cell Physiol.,* 24: 139-142.

Lee YI and Lee N (2003). Plant regeneration from protocorm-derived callus of *Cypripedium formosanum*. *In Vitro Cell. Dev. Biol.-Plant,* 39: 475-479.

Li XL and Wang XM (2008). Study on the rapid-propagation technical system with the petioles of *Pinellia ternate* Breit as explants. *Journal of Anhui Agricultural Sciences,* 36(6): 2247-2248.

Luo CK and Peng ZS (2005). Induction of pultaceous callus in *Pinellia ternate* (Chinese). *Journal of China West Normal University (Natural Science),* 26(1): 37-43.

Luo GM, Liu XW, Yao ZS, Lai XW and Ge F (2000). Tissue culture and regeneration of *Pinellia ternate* (Chinese). *Journal of Jiangxi College of Traditional Chinese Medicine,* 12(3): 125-126.

Makunga NP and Staden JV (2008). An efficient system for the production of clonal plantlets of the medicinally important aromatic plant: *Salvia africana-lutea* L. *Plant Cell Tiss Organ Cult.,* 92: 63-72.

Murugesan D, Doo HK and Seung-Beom H (2008). Efficient plant regeneration via somatic embryogenesis and organogenesis from the explants of *Catharanthus roseus*. *In Vitro Cell. Dev. Biol.-Plant,* 44: 18-25.

Mao ZC and Peng ZS (2003). Progress on research of rapid propagation system of *Pinellia ternate* (Chinese). *China Journal of Chinese Materia Medica,* 28(3): 193-195.

Murashige T and Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant,* 15: 473-497.

Peng ZS, Luo CK, Cai P, Mao ZC, Kang CY and Yang J (2007). Rapid propagation of the medicinal plant *Pinellia ternate* by in vitro leaves culture. *Bulgarian Journal of Agricultural Science,* 13: 1-6.

Ren JH, Chen KR and Xu QF (1983). Organ induction of trefoil *Pinellia ternate* (Chinese). *Plant Physiology Communications,* (4): 44-45.

Shoyama Y, Hatano K and Nishioka I (1983). Clonal Multiplication of *Pinellia ternate* by Tissue Culture. *Planta Med.,* 49(1): 14-l6.

Wang JL, Liao XR, Zhang HM, Du JF and Chen PL (2003). Accumulation of chlorogenic acid in cell suspension cultures of *Eucommia ulmoides* Oliv. *Plant Cell Tiss Organ Cult.,* 74(2): 193-195.

Xu XM, Lu DX, Sun M, Zhang M and Chen M (2005). Clustered shoot induction and rapid propagation on *Pinellia ternate* (Chinese). *Journal of Chinese Medicinal Materials,* 28(12): 1052-1053.

Xue JP, Zhang AM, Ge HL and Sheng W (2004). Technique on artificial seeds of *Pinellia ternate* (Chinese). *China Journal of Chinese Materia Medica,* 29(5): 402-405.

Yu Y and Wei ZM (2008). Increased oriental armyworm and aphid resistance in transgenic wheat stably expressing Bacillus thuringiensis (Bt) endotoxin and Pinellia ternate agglutinin (PTA). *Plant Cell Tiss Organ Cult.,* 94: 33-44.

Zeng LB, Zhou JY, Zhao J and Xiong L (1995). Effects of different plant growth regulator on morphogenetic in tissue culture of *Pinellia ternate* (Chinese). *Journal of Anhui Normal University,* 18(3): 42-46.

Zhang AM, Yang SY, Xue JP, Sheng W, Gao X and Zhu YF (2005). Effects of different factors on direct induction of microtubers from explants in *Pinellia ternate* (Chinese). *China Journal of Chinese Materia Medica,* 30(8): 576-579.

Zhang XW, Wang XF and Zhang XC (2006). Research advances on *Pinellia ternate* (Chinese). *Research and Practice on Chinese Medicines,* 20(6): 57-61.