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Rapid Propagation of the Medicinal Plant *Pinellia ternata* by *in vitro* Leaves Culture

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Abstract

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Pinellia ternata (Thunb) Breit is an important medicinal plants. Its tubers,being called as Banxia in Chinese words, were a crude drug of traditional Chinese medicine and some pharmaceutical products. For satisfying the demand of the drug market, some Chinese farmers began to cultivate this species in the field, and some breeders are work hard to domesticate this species into a crop. Dislike that of normal crops, the propagation rate of *P. ternata* is very low in nature condition. This study introduced a rapid propagation method by direct organogenesis of *Pinellia ternata* plantlets. This method used *Pinellia ternata* leaf as explants, and the regenerated *in vitro* plantlets obtained directly on the MS medium supplemented with the 0.5 mg/l benzyladenine and 0.5 mg/l α-naphthalene acetic acid. After transplanted into soil, the *in vitro* plantlets themselves showed rapid growing and high nature propagation rate. In addition, the tubers of *in vitro* plants much less polluted by heavy mental such as plumbum, cadmium, hydrargyrum and arsenic, after a session growing in soil.
Key words: Pinellia ternata, *in vitro* plantlet, medicinal plant, rapid propagation

Introduction

Plants are an important source of medicines and play a key role in word health (Constable, 1990). And medicinal plants are important to global economy (Srivastava et al., 1995). *Pinellia ternata* (Thunb.) Breit (Araceae)is one of this kind of medical plants. Its tubers,being called as Banxia in Chinese words, were a crude drug of traditional Chinese medicine and some pharmaceutical products. The tubers of this species were also used as crude drug in Korea, and being named as Ban Ha in Korean (Chung et al 2002). It has the medicine effective of anti-emetic, anti-inflammatory, sedative, antitussive and expectorant (Kurata et al., 1998, 1999).

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In addition, it is also used as food with special taste after treatment in some area of China. This species is distributed mainly in China, and also in Korea and Japan. It is a perennial shade plant species. Most wild plants of this species grown in crop farm, and be considered as weed. For the widely usage of herbicide in modern agricultural production, these wild plants were massacred. And the volume of usage in medicine compelled people to over-gather *P. ternata tubers* in the wild. Now, the wild resource of *P. ternata* is over exploited. But the Banxia trade is a big business between and among the East Asian countries (Chung et al., 2002). For satisfying the demand of the drug market, some Chinese farmers began to cultivate this species in the field, and some breeders are work hard to domesticate this species into a crop. Dislike that of other weeds and normal crop plants, the propagation rate of *P. ternata* is very low in nature condition. *P. ternata* plants mainly depended on their bulbils for asexual propagation, and a mature plant grow only 7-8 bulbils per year. Its sex reproduction was degenerated,showing a low rate of seed set and very poor seed germination (Peng and Cheng 2006, Wang et al., 2001). This shortage perplexes the farmers. To solve this problem, a tissue culture method was induced to accelerate *P. ternata* propagation in this investigation.

Material and Methods

Pinellia ternata plants were collected in Nanchong City, Sichuan, China, where is the main production area of this species and wild plants growing in the crop farm. Before being used in this research, it was cloned by the asexual propagating three generations in the experimental field under normal cultivated condition.

Plant material sterilization. The tubers of *Pinellia ternata* plants were sowed in the field, and the laminae of young leaves (about 3-4 cm long) were serviced as source of explants. The young laminae were excised from mature donor plants and washed with running tap water, soaked in 70% ethanol (v/v) for about 30 seconds, followed by immersion in 0.1% $HgCl_2$ for surface-disinfecting under continuous agitation for 10 min. they were subsequently rinsed three times with sterile distilled water.

Tissue culture. The sterilised leaves were cut transversely into approximately 5 mm x 5 mm pieces explants. Then the leaf explants were inoculated in 150-ml glass jars (five explants per jar) containing MS (Murashige and Skoog, 1962) medium supplemented with phytohormones. The medium was adjusted to pH 5.8 before autoclaving at 121 ^{o}C for 15 min under a pressure of 1.05kg cm^{-2}. The basal surface of leaf segments was kept in contact with the medium. The cultures were placed on a culture room at 25±2 ^{o}C under lighting with cool white fluorescent tubes at an intensity of about 2500 lx. The culture program maintained at a light and dark interval of 12-h photoperiod.

Transfer to soil. For acclimatization, regenerated plantlets with abundantly developed leaves, tubercle and roots were transferred to pots containing normal soil from the experimental field. The regenerated in vitro plantlets were covered with a polyethylene membrane to maintain high humidity during the first week, and cultured at nature temperature and light conditions. Then the polyethylene membrane was gradually opened. The plantlets were irrigated with tap water and managed as those in the experimental field.

The tubers were analysis by using the Atomic Absorption Llame Emission Spectrphotometer (Model AA 6701, made in Japan) to determine the contents of plumbum and cadmium. And Atomic Fluorescence Spectrograph (Model AF 610, made in China) was used to determine their hydrargyrum and arsenic content.

Results and Discussion

The organogenesis of *Pinellia ternata* in culture was affect by the phytohormones' combination and sorts. If the explants were cultured on MS medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D), the callus would foremost appeared, and then plantlet differentiated from these callus after being transferred into differential medium. If there is no 2,4-D in the medium, no visible callus appeared at all, and the plantlets directly developed from the explants without being transferred into differential medium, which was called as direct organogenesis. According to the result of previous experiment in our laboratory, the MS medium supplemented with 0.5 mg/l benzyladenine is suitable to tissue culture of *Pinellia ternata.* In this study, the effect of α-naphthalene acetic acid (NAA) on the direct organogenesis of *Pinellia ternata* was investigated. And the results were listed in Table 1. When no NAA existed in the MS medium, the differentiation frequency was very low (4%) and only 3 tubercles differentiated per explant. When the concentration was 0.1mg/l, both the percentage of explants developing tubercles and percentage of explants developing tubercles were over 60%. NAA could significantly raise the differentiation frequency. But too high concentration did not raise the culture effect, and on the contrary decrease it. When considering the number of explants formed tubercles and the number of explants formed roots, the 0.1-1.0 mg/l NAA are better. But in the medium containing 1.0 mg/l NAA, the number of differentiated tubercles per explants is just 6.54, much lower than the medium containing 0.1or 0.5 mg/l NAA. So, the concentration of NAA was suggested 0.1and 0.5 mg/l in practice, especially the 0.5 mg/l NAA is the best one. The in vitro pantalets in the following study were obtained with MS medium supplemented with 0.5 mg/l NAA and 0.5 mg/l benzyla-denine but without 2,4-D.

After 7 days *in vitro* cultured, the leaf

Table 1

Effect of NAA on the direct organogenesis of *P. ternata in vitro* plantlets

Concentration of NAA, mg/l	Percentage of explants developing tubercles	Percentage of explants developing roots	Number of tubercles per explants
0	4%	4%	3.00
0.1	62%	61%	8.99
0.5	76%	72%	9.97
1.0	70%	70%	6.54
2.0	46%	36%	6.76
4.0	48%	26%	5.94

explants showed some morphology changes. The explants humping up and the incision part began expanding and its green colour fading. Up to 28 days, no callus was observed by eye seeing, but some small white tubercles appeared in the incision parts of the explants. Then these tubercles grown bigger and bigger, and were turned to green and green. Latterly, the similar tubercles were observed in other parts of the explants. even almost all of the explants surface could be covered by tubercles. At last, white roots were appeared below tubercles (Figure 1). About 60 days, the abundantly developed regenerated in vitro pantalets with green new leaf on the tubercles were obtained (Figure 2), when the tubercle should be called as tuber of the plantalet. These regenerated in vitro pantalets would separate each other spontaneously, and we can easily get individual regenerated *in vitro* pantalets for transplanting.

After transplanting to soil in pots, the regenerated *in vitro* pantalets would change morphologically. All the new *in vitro* pantalets carried one or more cordate or auriculate leave as showed in Figure 2. *P. ternata* plants caring only this kind of leave could not produce any new bulbils, which was similar to those grown in wild condition as revealed previously (Peng and Cheng 2006). About two months later, the *in vitro* pantalets could grow normal compound leaves with three foliole, and the in vitro pantalets could be named as *in vitro* plant because no visible morphological difference existed between them and the plants from nature propagation. This leaf morphology change was similar to that of young plants deriving from bulbils in wild condition, but the plants from normal bulbils in nature propagation need at least one full year to complete the course *in vitro*.

In this investigation, the *P. ternata* in vitro plants with compound leave could grow bulbils (Figure 3) that were the main agamonts of this species. Nevertheless, the compound leave just developed from two

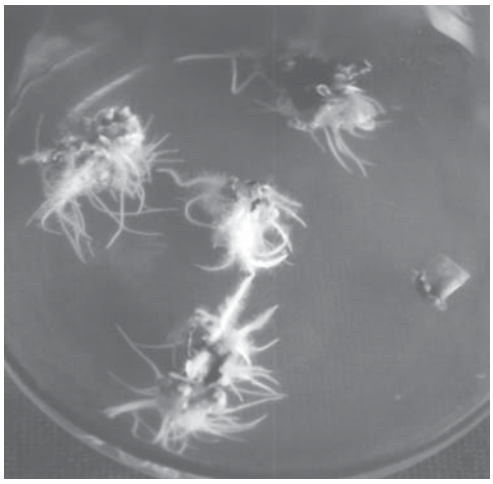

Fig. 1. The explants of *P. ternata* in culture, showing the green tubercle ans white roots

Fig. 2. The *P. ternata* *in vitro* plantlets with green leaves and white roots in the jar

Fig. 3. The mature plants from *in vitro* plantletsq showing the compound leaves and brown bulbils (rotundity shape ones)

years old plants in natural propagation (Peng and Cheng, 2006). The bulbils in the *in vitro* plants were smaller than those from nature propagation, as the in vitro plants were still small at that time. But they could develop into normal plants too. All of the in vitro plants developed bulbils, with one in vitro plant growing 12.8 bulbils in mean. Considering that one plant from nature propagation grow only 7.8 bulbils in mean as reported by Pan (1998), the *in vitro* plants could be considered having higher propagation rate than the nature ones.

After four to five weeks in the soil, some *in vitro* plants emergent inflorescence that was called as spadix to P. ternata. Only 16.7% *in vitro* plants emergent inflorescence at the fist year. As those from nature propagation, the spadix of *in vitro* plants was rarely seed set, and a fat lot of seeds can germinate.

The tuber morphology of these in vitro plants was normal. But its growth rate was much higher than nature propagated plants. When transplant to soil, the tubers were only 3.0 mm in diameter. The tubers rapidly grown much big, their size were up to 22 mm in diameter after one season in soil. But the normal plants would spend over 2 years to develop the same size of tubers.

After one session of growth in the soil, the heavy metal content of the tubers from *in vitro* plants was determined, and the result listed in Table 2. The investigated heavy metals included plumbum, cadmium, hydrargyrum and arsenic. The tubers from *in vitro* plants carried very low heavy metal content. And the content of each metal of the tubers derived from *in vitro* plants was lower than the same sizes of tubers derived from normal plants. This result means that the *in vitro* plants were much less populated. What cause this circumstance was not surely known. It may be due to their shorter time in soil than the same size of tubers from normal plants, for they grown much faster.

Table 2

The heavy metal content (mg) of 1 kg dry tubers derived from *in vitro* plants and normal plants

Heavy metals	Plumbum (Pb)	Cadmium (Cd)	Hydrargyrum (Hg)	Arsenic (As)
Tubers from *in vitro* plants	0.153	0.238	0.012	0.044
Tubers from normal plants	0.58	0.39	0.018	0.091

Conclusion

To accelerate of rapid propagation of *P. ternata*, the tissue culture method (Shoyama, 1983; He, 1996) and artificial seeds technique (He et al., 1997; Xue et al., 2004) have been previously induced by scientists. But there is no one been used in practice up to now, because the farmers considered these methods greatly increase the production cost (Mao and Peng, 2003). This study reported a new tissue culture method resulting in direct organogenesis of *P. ternata* plantlets. By this method, both the differential medium and the corresponding culture course were omitted. So, it could save time and reagent, which make the cost of the rapid propagation cheaper.

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