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Study on Tissue Culture and Rapid Propagation of *Platycodon grandiflorus*

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Abstract With seeds as experimental materials, MS was used as the basic medium to combine different species and concentrations of auxin and mitogen for proliferation, elongation and rooting culture. The best combination of medium and the most suitable medium were selected. The results showed that the best formula for the medium was MS + 0.5 mg/L BA + 0.5 mg/L IAA + 30 g/L sucrose in the proliferation culture, MS + 0.25 mg/L BA + 0.5 mg/L IAA + 30 g/L sucrose in the elongation culture, and MS + 0.5 mg/L IAA + 20 g/L sucrose in the rooting culture respectively. The experimental results will be applied in the rapid propagation and breeding of high-quality seedlings of *Platycodon grandiflorus*.

Key words *Platycodon grandiflorus*, Medium, Hormone, Proliferation culture, Differentiation

1 Introduction

Platycodon grandiflorus (Jacq.) A. DC. is a perennial herb belonging to the family Campanulaceae. Its roots can be used as medicine and have the functions of moistening the lungs, dispelling cold, eliminating phlegm and expelling pus. Its flowers are big, beautiful and colorful and have ornamental value, so it is a promising ideal herbaceous flower. The flowers are mostly blue, purple or white. The gestures are light, and the colors are fresh and elegant. The flowers are unique and lovely in shape, and it is one of the most popular potted flowers and cut flowers in the world[1]. As a garden plant, it is also loved by people because it is quiet and beautiful, quietly elegant and solemn, and its ornamental value is high. For landscaping or decorating parks, it is a very good flower variety when being planted around rockery and in flower beds and flower borders or used in large-scale celebrations[2].

P. grandiflorus is distributed in China, the Korean Peninsula, Japan and eastern Siberia. It blooms in late summer and early autumn and can be cultivated on a large scale as an ornamental flower[3]. The yield and quality of its seeds are unstable and expensive. Its wild resources are scarce, and the breeding of its seedlings take a long time and is costly[4–5]. In this study, the seeds of *P. grandiflorus* were used for tissue culture, and the rapid propagation system of the initial culture was established. The effects and the best ratio of plant hormones in the rapid propagation process were studied to provide scientific reference for shortening the breeding period of seedlings and rapid propagation of fine varieties of *P. grandiflorus* and promoting the cultivation of cut flowers and large-scale production of seedlings of *P. grandiflorus*.

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2 Test materials and methods

2.1 Test materials The seeds of *P. grandiflorus* used in the test were provided by the Garden Plant Laboratory of the Department of Horticulture, Tianjin Agricultural University.

2.2 Test methods The seeds were washed with distilled water firstly and then soaked in 75% alcohol for 30 s. Afterwards, they were disinfected with 40% sodium hypochlorite solution for 5 – 7 min and washed with sterile water 3 – 5 times. After being dried with sterile filter paper, the washed and sterilized seeds were inoculated onto MS media. Each culture flask had 10 – 12 seeds. The seeds were cultured at 25℃ under 24-h light.

When the seeds germinated and grew to 2 – 3 cm, the seedlings were cut and transferred to a medium containing different growth hormones for the proliferation culture of the regeneration buds. In the proliferation culture, 100 mL wide-mouth flasks were used, in which 35 mL of a solid medium was put. The formulas for the proliferation media are as follows: first, 0.5 mg/L IAA was combined with 0.25, 0.5, 1.0 and 1.5 mg/L BA respectively; second, 0.5 mg/L IAA was combined with 0.25, 0.5, 1.0 and 1.5 mg/L KT respectively. There were 8 flasks in each treatment, and 5 axillary buds of *P. grandiflorus* were inoculated in each flask. After they were cultured at 25℃ under 24-h light for 40 d, the proliferation situation of the axillary buds in each treatment was counted.

As the buds of *P. grandiflorus* grew to 2 – 3 cm, they were separated from the buds on a clean bench, and each individual bud was inoculated separately onto MS media containing different hormones for the elongation culture of the regeneration buds. The containers, medium dosage and culture conditions adopted in this experiment was the same as the last experiment. The formulas for the elongation media are as follows: first, 0.25 mg/L IAA was combined with 0.10 and 0.15 mg/L BA respectively; second, 0.5 mg/L IAA was combined with 0.25 and 0.5 mg/L BA respectively; third, 1.0 mg/L IAA was combined with 0.75 and 1.0 mg/L BA respectively. 8 – 10 regeneration buds were inocu-

lated in each treatment, and each treatment had five repetitions. After 30 days of culture, the elongation situation of the regeneration buds in each treatment was counted.

When the seedlings of *P. grandiflorus* grew to 5 cm during the process of elongation culture, they were transferred to a medium containing different growth hormones for the rooting culture of the regeneration seedlings. The formulas for the rooting media are as follows: first, 1/2 MS was combined with 0.25, 0.5, 1.0 and 2.0 mg/L IAA respectively; second, MS was combined with 0.25, 0.5, 1.0 and 2.0 mg/L IAA respectively. 5 – 8 regeneration seedlings were inoculated in each flask, and it was repeated 5 times. The culture conditions were the same as before. After 30 days of culture, the rooting situation of the regeneration seedlings in each treatment was counted.

3 Results and analysis

3.1 Proliferation situation of axillary buds of *P. grandiflorus* After 40 days of proliferation culture, the number of the proliferating buds varied somewhat between different plant hormones and different concentrations of media. According to Table 1, the proliferation coefficient obtained by using BA as a mitogen was significantly higher than that of KT. Seen from the growth situation of the regeneration buds, the regeneration buds induced by BA were stronger. However, the regeneration buds induced by KT grew slowly, and there existed vitrification phenomenon, showing that KT was not conducive to the proliferation culture of *P. grandiflorus*. There were also differences between different concentrations of the same mitogen in terms of proliferation situation of *P. grandiflorus*. When BA was used as mitogen, the proliferation coefficient increased firstly and then reduced with the increase of its concentration. As KT concentration increased to 0.5 mg/L, the regeneration buds grew fastest, and the proliferation coefficient was the highest. The results indicate that the proliferation effect of *P. grandiflorus* was the best under the action of suitable BA. To sum up, the MS medium containing 0.5 mg/L BA, 0.5 mg/L IAA and 30 g/L sucrose was the fittest for the proliferation culture of *P. grandiflorus*.

Table 1 Proliferation situation of axillary buds of *Platycodon grandiflorus*

No.	Concentration // mg/L			Number of inoculated buds	Number of proliferation buds	Proliferation coefficient	Growth situation of proliferation buds
	BA	IAA	KT				
1	0.25	0.5	0	50	90	1.8	Grade 3
2	0.50	0.5	0	48	157	3.3	Grade 3
3	1.00	0.5	0	45	146	3.2	Grade 2
4	1.50	0.5	0	51	114	2.2	Grade 2
5	0	0.5	0.25	55	61	1.1	Grade 1
6	0	0.5	0.50	45	58	1.4	Grade 1
7	0	0.5	1.00	40	40	1.3	Grade 1

Note: In the table, MS was used as the basic medium, to which certain kinds of hormones and 30 g/L sucrose were added. Proliferation coefficient = number of proliferation buds/number of inoculated buds. The growth situation of proliferation buds were divided into grades 1 (growing slowly and appearing vitrification phenomenon), 2 (growing relatively normally), and 3 (growing normally and strongly). The same as below.

3.2 Elongation situation of regeneration buds of *P. grandiflorus* Seen from Table 2, the differentiation of *P. grandiflorus* mainly depended on the ratio of the mitogen to the auxin. When the ratio was high, the regeneration buds remained in a differentiation state, and new regeneration seedlings grew, while the original regeneration buds therefore could not stretch. If the cultivation time was extended, the regenerated buds would be vitrified, browned and dead, and the vigorous seedlings could not be obtained. As the ratio decreased, the phenomenon of differentiation was inhibited, and the regeneration seedlings elongated and grew fast. In a word, if the ratio of the mitogen to the auxin was appropriate, the elongation effect of the regeneration seedlings was good. When the regeneration buds of *P. grandiflorus* were cultured in the MS medium containing 0.25 mg/L BA and 0.5 mg/L IAA, the elongation effect was the best.

Table 2 Elongation situation of regeneration buds of *Platycodon grandiflorus*

No.	Concentration // mg/L		Number of inoculated buds	Growth length // cm	Growth effect
	BA	IAA			
1	0.10	0.25	40	1.7	Grade 1
2	0.15	0.25	40	2.1	Grade 1
3	0.25	0.50	40	3.4	Grade 3
4	0.50	0.50	40	1.9	Grade 1
5	0.75	1.00	40	2.4	Grade 2
6	1.00	1.00	40	1.3	Grade 1

3.3 Rooting situation of tissue culture seedlings of *P. grandiflorus* IAA with lower osmotic pressure was used as the basic medium, to which 20 g/L of sucrose was added. Although 1/2 MS medium could make tissue culture seedlings root, the number and length of the roots were small. Seen from the quality of the roots, the roots induced on 1/2 MS medium were short and thick fleshy roots, and did not have any physiological functions of true roots, so such tissue culture seedlings generally could not survive. The roots induced on MS medium were normal and strong, and the number and length of the roots were very ideal. When MS medium was used to induce rooting, a larger number of regeneration roots could be induced with the increase of IAA concentration. As IAA concentration reached 1.0 mg/L, the quality of the roots changed obviously, and the length of the roots reduced. Moreover, the roots were straight and thick, and there were few lateral roots. These roots were also similar to the fleshy roots induced by 1/2 MS medium, and the transplantation survival rate was low. In a word, when the seedlings of *P. grandiflorus* were cultured in

the MS medium containing 0.5 mg/L IAA and 20 g/L sucrose, the rooting effect was ideal.

Table 3 Rooting situation of tissue culture seedlings of *Platycodon grandiflorus*

Formulas for the media	Average quantity of roots	Average length of roots	Shape of roots
1/2 MS + 0.25 mg/L IAA	2.2	2.1	Thin hairy roots
1/2 MS + 0.5 mg/L IAA	3.8	3.2	Thin hairy roots
1/2 MS + 1.0 mg/L IAA	3.9	1.9	Thin hairy roots
1/2 MS + 2.0 mg/L IAA	1.5	1.7	Thin hairy roots
MS + 0.25 mg/L IAA	4.6	4.3	Normal healthy roots
MS + 0.5 mg/L IAA	7.1	4.8	Normal healthy roots
MS + 1.0 mg/L IAA	5.2	3.2	Short clustered roots
MS + 2.0 mg/L IAA	3.1	3.1	Short clustered roots

4 Conclusions and discussions

Wang Guimei *et al.* induced the callus of stem segments as explants on the MS medium containing different concentrations of plant hormones to select the best formula for the medium, and found that the MS medium containing 1.0 mg/L 6-BA and 0.5 mg/L NAA was most propitious to the induction of the callus[5]. Zhu Yuling proposed that the best formula for the medium used to induce stem tips was MS + 0.2 mg/L NAA + 0.8 mg/L 6-BA[6]. In this experiment, the MS medium containing 0.5 mg/L BA and 0.5 mg/L IAA was the fittest for the proliferation culture of stem tips of *P. grandiflorus*. The results showed that the effect of BA was much better than that of KT, it is more suitable to use BA to induce *P. grandiflorus*. In tissue culture, the effect of BA on herbaceous plants is generally better than KT, because herbs are more sensitive to hormones, and high concentrations of hormones may have toxic effect on them. Therefore, in actual induction culture, when a hormone with a strong effect, like KT, is used, the growth of explants is inhibited due to the toxic effect of KT, thereby affecting the actual proliferation effect. In this experiment, under the action of KT, the growth of explants of *P. grandiflorus* was inhibited, and the differentiation effect was poor. Seen from the experimental results, in tissue culture, it is necessary to select hormones reasonably and adjust the concentration appropriately to obtain the desired test results.

In the elongation culture of tissue culture seedlings of *P. grandiflorus*, different proportions of auxin and mitogen were used. The results showed that the elongation of *P. grandiflorus* could be promoted if the ratio of hormones was appropriate, and the MS medium containing 0.25 mg/L BA and 0.5 mg/L IAA was the best. A study showed that with the increase of 6-BA concentration, the proliferation rate of a single bud of *Eustoma grandiflorum* (Raf.) Shinners increased, and the proliferation coefficient decreased when the concentration reached a certain value; the MS medium containing 0.2 mg/L 6-BA and 0.1 mg/L NAA was the most ideal proliferation medium[7]. In the study, when the ratio of mitogen to auxin was high, the plants grew slowly, and the proliferation phenomenon still continued. As the ratio decreased, the phenomenon gradually changed, and the regeneration seedlings gradually made the transition from the proliferation state to the rapid growth state. In the proliferation culture, the scheme for the elongation culture was determined according to the growth condition of the specific regeneration seedlings.

In the rooting culture of tissue culture seedlings of *P. grandiflorus*, a certain concentration of auxin was used to stimulate rooting, and the method of reducing osmotic pressure was also used to promote the elongation of the regeneration roots. A report revealed that the induction rate of roots of *E. grandiflorum* seedlings cultured in 1/2 MS was low, and the higher the NAA concentration was, the more the induced roots were[8]. The experimental results showed that under the action of 0.5 mg/L IAA, the rooting effect was the best when MS was used as the basic medium, which was different from the results of Zhu Yuling *et al.* (the best rooting medium was 1/2 MS + 0.2 mg/L NAA) and Yang Yaowen *et al.* (rooting was fast when the medium was 1/2 MS + 0.5 mg/L NAA + 0.1mg/L IAA[9]. According to the growth of *P. grandiflorus*, *P. grandiflorus* is a plant species with weak growth vigor, and in the growth culture of tissue culture seedlings, the growth of the plants was obviously slowed down when 1/2 MS was used. The plants were weak and generated weak hairy roots due to the deficiency of plant nutrients. As MS medium was used, the phenomenon was restored, so the effect of MS in the rooting culture of *P. grandiflorus* was better than that of 1/2 MS.

Research on the tissue culture technology of *P. grandiflorus* is still in the development stage. The experimental results of this study contribute to further research and exploration in this field. The experiments proved that using seeds as explants to culture tube seedlings could shorten the breeding period of *P. grandiflorus* seedlings and ensure excellent varieties, providing some data for the cultivation of cut flowers and large-scale cultivation of seedlings.

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