J. Life Science. (2002), 12(1), 19~21

JOURNAL OF
Life Science
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Effect of 2,4-D on embryo formation and its morphology in anther culture of herbaceous peony (*Paeonia lactiflora* Pall.)

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Received: April 29, 2002

Abstract The pathway of embryos formed anther culture in herbaceous peony was influenced by addition of 2,4-D. MS medium with 2,4-Dichlorophenoxy acetic acid (2,4-D) alone did not arise direct embryogenesis, but was proliferate callus. Embryos through calli were produced on medium containing 0.2 mg/l zeatin or without growth regulators. Direct embryogenesis was obtained from MS basal medium. However, after the anthers were cultured on medium with 0.1 mg/l 2,4-D, 3 g/l AC, 30 g/l sucrose, 2 g/l gelrite for 40 days. Its efficiency (32.3%) was markedly improved when anthers cultured on medium without 2,4-D. Embryo morphology was also affected by the 2,4-D used in medium. The induction of normal embryos with two cotyledons was higher in the embryos formed through direct embryogenesis than those formed callus. The embryos formed from calli were mainly showed abnormal embryo with one, three, four cotyledons or horn and bowling pin type.

Key words: *Paeonia lactiflora* Pall., embryogenesis, anther culture, two-cotyledon embryo

Introduction

Herbaceous peony (*Paeonia lactiflora* Pall), which belongs to perennial herb, has been cultivated as a medical plant since ancient time in Korea, China, and Japan etc. In recent year, it was widely increased cultivation area for medical plant in Korea. For anther culture of *Paeonia* species, rate of growth in culture is low: anther-inoculation to plant emergence take about 4 to 6 month [9]. Haploid callus of herbaceous peony was obtained from medium containing various growth regulators [7]. Sunderland [10] reported that the peonies vary in frequency between species, between varieties of the same species and between anthers of the same plant. Despite the attempts to study an anther culture in Korea, such as the initial division of microspores [2], cold temperature treatment [5], and pollen dimorphism and growth regulators [4], haploid production is commonly obtained at too low efficiency. In this report, we describe to improve the frequency of haploid production and the influence of 2,4-D on morphology of embryo being produced from anthers.

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Materials and Methods

Plant materials

The 'Euseongjagyag (*Paeonia latiflora* Pall.)', a herbaceous peony which is cultivated in Korea, was used as experiment plant materials and flower buds were collected from the field in May 1993. Flower buds were harvested when most of the microspores were uninucleate, which is normally reached when the buds are 15~20 mm, and then, pretreated for 10 days at 5℃. They were disinfected surface with a 70% ethanol for 30 sec. and were rinse several times with sterile distilled water. The flower buds were separated with forceps and anthers dissected out.

Embryo formation

Twenty anthers were cultured on MS medium [6] containing 30 g/l sucrose, 2 g/l gelrite in a petridish (Φ90 mm). Callus induction was initiated from anther cultured on different concentration of 0.1, 0.5, 1.0, and 2.0 mg/l 2,4-D for 40 days. Embryo formation have counted since calli were transferred on MS containing 0.2 mg/l zeatin. The ruptured anthers were compared to MS medium with 2,4-D alone at concentration of 0 or 1.0 mg/l 2,4-D at concentration of 0.1, 1.0 mg/l in combination activated charcoal (AC) at 3 g/l. The ruptured anthers were transferred to MS hormone free medium and investigated to embryo formed through direct embryogenesis.

Plant regeneration

Embryos formed anther culture were precultured for 20 days on MS containing 0.3 mg/l GA_3. The germinated embryos were treated to 4℃ in dark condition for 8 weeks and developed to MS hormone free medium at 25℃ with 16/8 h day/night. Plant with developed root systems was harden off Jiffy pots (Φ9 cm) containing a vermiculite in a growth chamber at 25℃. Plants were grown in the same conditions the mother plants (Fig. 1-C).

Embryo morphological classification

After 45 days on each of embryo formation medium, embryos were categorized into 6 developmental classes based on cotyledon number and morphology.

Results and Discussion

The effect of 2,4-D on callus and embryo formation was investigated (Table 1). The addition of 2,4-D slightly increase the callus induction. However, the highest of embryo forming response, more than 13%, was observed from callus induction medium supplemented with 1.0 mg/l 2,4-D.

The using of 2,4-D alone or 2,4-D and AC was investigated to effect of embryo formation (Table 2). In the case of 2,4-D alone, the culture of low 2,4-D concentration (0.1 mg/l) results in a significant reduction of embryos on a direct embryogenesis, whereas a callus was promoting. In contrast, the pollen-derived embryos producted directly from medium devoid of 2,4-D or 2,4-D and AC without the formation of a callus. The combination of 0.1 mg/l 2,4-D and 3 g/l AC induced high frequency (32.3%) of embryo formation.

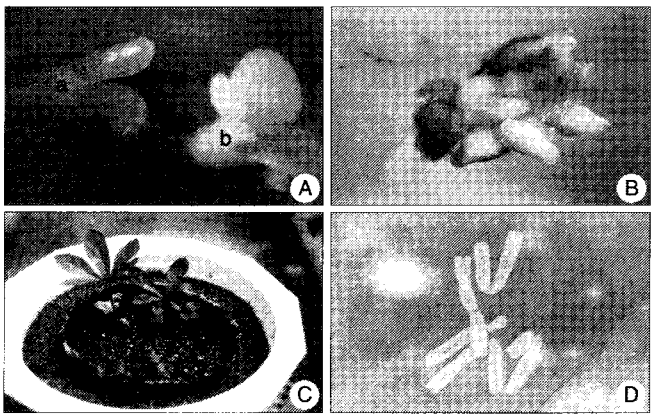

Fig. 1. Haploid production and morphological variation of pollen-derived embryo in *Paeonia lactiflora* Pall. A: Two (a) and three (b) cotyledonary embryo. B: Abnormal embryo formed from callus. C: Plants transplanted in potting vermiculite. D: Chromosome of haploid plant.

According to embryo morphology (Table 3), the pollen-derived embryos were related to 6 classes, which mainly differed in embryos of cotyledon number and morphology. The maximum frequency (84.1%) of normal embryo with two cotyledon (Fig. 1-Aa) was obtained from embryos formed through direct embryogenesis. These most closely resembled zygotic embryo morphology. In contrast, addition of 2,4-D at 1 mg/l in anther cultures was not direct embryogenesis, but promoted callus. When the calli were transferred to MS medium with zeatin at 0.2 mg/l (data not shown) or without growth regulators, proportion (5.9~27.5 %) of abnormal embryo (Fig. 1-B) with one, three (Fig. 1-Ab), four cotyledon umber or bowling pin and horn type.

In this report, direct embryogenesis did not produced further if maintained in induction media containing 2,4-D alone. The period of exposure to 2,4-D improved callus proliferation. However, the combination of 2,4-D and AC resulted the highest percentage of embryo formation from anther. It is conjectured that the use of AC was the adsorptive function of the 2,4-D from medium. Sohn and Kim [8] reported that best were obtained form the callus formed in the presence of 1.0 mg/l 2,4-D and 0.2 mg/l NAA. This report found that the addition of 2,4-D was enhanced to callus formation, but the production of embryos was optimum when 1.0 mg/l 2,4-D was utilized. Direct embryogenesis from anther occurred to medium without growth regulators. This concurs with Sunderland [9,10] who found that MS basal medium obtained to embryo production in anther culture of peony.

2,4-D has been reported to affect both frequency of somatic embryo and morphology in plant tissue culture. In immature embryo culture of soybean (*Glycine max*), addition of 2,4-D markedly increased to abnormal embryo, compared to NAA [3]. Choi et al. [1] observed that abnormal embryo was induced more the high level of 2,4-D than that of low for soybean embryo cultures. In this study, the induction frequency of normal embryos with two cotyledon was about two times higher in the embryo formed through direct embryogenesis than those formed callus. We have critically conclude that abnormal embryo with one, three, four cotyledon or horn and bowling pin type induced by 2,4-D.

Tbale 1. Effect of 2,4-D on callus and embryo formation in anther culture of *P. lactiflora*

2,4-D (mg/l)	No. of anthers cultured	No. of anthers forming callus (%)	No. of calli transferred	No. of callus forming embryo (%)
0.1	320	48 (15.0)	90	2 (2.2)
0.5	280	50 (17.9)	100	4 (4.0)
1.0	880	228 (25.9)	450	60 (13.3)
2.0	380	155 (40.8)	130	12 (9.2)

Table 2. Effect of 2,4-D and activated charcoal (AC) on embryo formation of anthers ruptured in anther culture of *P. lactiflora*

Plant hormone and addition		No. of anthers cultured	No. of ruptured anthers transferred	No. of anthers forming	
2,4-D (mg/l)	AC (g/l)			embryo (%)	callus (%)
0	0	380	90	9 (10.0)	-[a]
1.0	0	300	90	4 (4.4)	21 (23.3)
0.1	3	399	164	53 (32.3)	-
1.0	3	239	80	17 (21.3)	-

[a]No forming callus.

Table 3. Morphological variation of embryos formed through direct embryogenesis and from callus in anther culture of *P. lactiflora*

2,4-D (mg/l)	No. of anther cultured	No. of forming embryo (%)	No. of total embryos	Percent of embryos				
				one-	two-	three-	four-cotyledonary	Bowling pin & horn type
0	720	80 (11.1)	131	7.3	84.1	6.6	0.7	1.3
1	760	140 (18.4)[a]	117	5.9	40.5	27.5	10.1	16.0

[a]Embryo formed from callus.

As a result of this study, if the frequency of haploid was in the increase. It is not only to shorten the breeding time but also study cytology by using haploid. Further study is needed to determine the method for chromosome doubling from these haploid plants.

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