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Callus Formation from Anther Culture in Balsam Pear (*Momordica charantia* L.)

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Abstract: The effects of microspore development stage, cold pretreatment, growth regulators and sucrose on anther culture in four cultivars of balsam pear (*Momordica charantia L.*) were investigated. Our objective was to identify a combination producing the maximum number of callus, which provided to regenerate plants. The results showed that the callus formation rate of anthers in late-uninucleate stage was higher than those in other microspore development stages. The best response of cv. Bixiu, Dabai and Pangniu anthers to *in vitro* culture was obtained when a 24h cold pretreatment was employed to flower buds at 4°C in darkness, while cv. Changbai presented a contrary result and fresh anthers formed more callus. Anthers on MS medium containing 3% sucrose, 2,4-dichlorophenoxyacetic acid (2,4-D) 0.5mg/L in combination with benzyladenine (BA) 2.0mg/L formed more and better callus.

Key words: Balsam pear (*Momordica charantia L.*) · Anther culture · Callus

INTRODUCTION

Balsam pear is an annual tendril herbage plant of balsam pear genus of gourd family. It is a kind of important and valuable vegetable crop and medicinal plant. There were few research on anther culture and only a few research concentrated on stem and cotyledon culture for fast propagation in balsam pear [1,2,3]. Anther culture was an efficient approach to obtain haploid plant and forming a mass of callus was the base of plant regeneration. The success of anther culture depends on numerous factors such as genotype, microspore development stage, cold pretreatment, concentration of growth regulators and sucrose in medium. The effects of these factors on anther culture in four cultivars of balsam pear were studied systematically.

MATERIALS AND METHODS

Plant Materials: Anthers of balsam pear cv. Bixiu, Dabai, Changbai and Pangniu were the experimental materials in the present investigations. The mother plants were grown in the experimental plots using standard agronomic practices.

Study on Relativity of Microspore Development Stage and Flower Bud Shape

Sampling: The flower buds of different size were took from balsam pear plants on 8:00 am to 10:00 am and immersed in Kano stationary liquid (alcohol : acetic acid= 3:1) for 12 h. Then the flower buds were transfer in 70% (v/v) alcohol solution. More than 10 flower buds of each size were studied.

Observation under Microscope: Flower buds in alcohol were blotted by absorbent paper and measured the length and breadth by ruler. The calyxes and petals were shucked off and anthers were put on microscopic slide, extruded by forceps gently, stained with one drop of Carbol-Fuchsin. After anther walls and residue removed, covered by cover slip, microslides were pressed and observed under microscope. Pollen was considered to be in a certain microspore development stage if the number of microspores in this stage was more than 50%.The relationship between length of flower buds, color of anthers and development stage of microspores were found.

Inducing Callus from Balsam Pear Anther

Method: All operations were carried out in a laminar air-flow cabinet under aseptic conditions. The flower buds were dipped in 75% (v/v) alcohol for 30 seconds, immersed in 0.1% (w/v) mercuric chloride solution with periodic agitation for 5minutes and washed with sterile distilled water for five times. After filament removed, the intact anthers were inoculated on inducing medium. Without special explaination, the flower buds were pretreated for 24 h under 4°C condition and medium

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consisted of MS mineral salts and vitamins, 5% (w/v) sucrose, 0.6% (w/v) agar and it was supplemented with growth regulators. The media were adjusted to pH 5.6 prior to addition of agar and sterilized at 122°C and 104kPa pressure for 20min. The anthers were cultured in a culture chamber at 25°C in the dark for 10 days and then at 25°C under 16h daily illumination with 1500lx fluorescent light.

Effect of Microspore Development Stage: Anthers of tetrad stage, early-uninucleate stage and late-uninucleate stage were inoculated on MS medium supplemented with 2,4-D 0.5mg/L and BA 2.0mg/L.

Effect of Cold Pretreatment: Anthers of the flower buds pretreated under 4¡æ condition for 0 h, 24 h, 48 h, 72 h, 96 h, 120 h were inoculated on MS medium supplemented with 2,4-D 0.5mg/L and BA 2.0mg/L.

Effect of 2,4-D and BA: Anthers were inoculated on MS medium supplemented with 2,4-D (0.1, 0.5, 1.0mg/L) and BA (0.5, 1.0, 2.0, 4.0mg/L).

Effect of Sucrose: Anthers were inoculated on MS medium supplemented with sucrose 0%, 3%, 5%, 7% (w/v).

Data Collection and Analysis: A randomized complete block design was used for callus induction experiments. 300 anthers were cultured (20 anthers per conical flask and 15 replicates per treatment). Cultures were recorded at regular intervals of 4 weeks. Significance between means was tested by Duncan's multiple range test.

RESULTS

Relativity of Microspore Development Stage and Flower Bud Shape: The relativity of flower buds and microspore development stage was summarized. When flower bud length was 2 to 3mm and anther was green, it was in tetrad stage (Fig. 1). When flower bud length was 3 to 4mm and anther was viridescence, it was in early-uninucleate stage (Fig. 2). When flower bud length was 4 to 5mm and anther was flaxen, it was late-uninucleate stage and when flower bud length was more than 5mm, it started binucleate stage, even became mature microspore (Fig. 3).

Effect of Microspore Development Stage on the Callus Formation from Anthers: Effect of microspore development stage on callus formation from anthers was statistically significant (Table 1). Apparently, the highest inductivity of callus was obtained when anthers in late-uninucleate stage, while the lowest occurred in anthers of tetrad stage. The callus formation rate of Bixiu reached 80.34% and it's the maximum.

Effect of Cold Pretreatment on the Callus Formation from Anthers: Analysis of variance of the callus induction rate indicated that callus formation was significantly affected by cold pretreatment. According to the data in Table 2, callus formation rate of Bixiu, Dabai, Pangniu increased after anthers pretreated under 4°C condition for 24 hrs. On the contrary, for Changbai, fresh anthers formed more callus than others. Anther callus of four balsam pear cultivars were hardly induced when pretreated for more than 96 h. When the cold pretreatment was up to 120h, no anthers formed callus and the anthers became necrotic within one week.

Table 1: Effect of microspore development stage on callus induction of anthers

	Callus formation rate (%)			
Microspore development stage	Bixiu	Dabai	Changbai	Pangniu
Tetrad	15.47c	17.52c	11.84c	15.35c
Early-uninucleate	54.74 b	66.86b	57.76b	55.48b
Late-uninucleate	80.34a	78.63a	78.26a	76.49a

Means having the same letter in the columns were not significantly different according to Duncan's multiple range test at P=0.05 (The same was the following tables).

Table 2: Effect of cold pretreatment on callus induction of anthers

	Callus formation rate (%)			
Pretreatment time(h)	Bixiu	Dabai	Changbai	Pangniu
0	40.26c	38.31c	80.55a	51.40b
24	73.16a	69.89a	60.32b	62.10a
48	60.54b	53.94b	51.79c	64.49a
72	18.27d	26.73d	19.22d	58.27ab
96	4.42e	2.54e	3.56e	6.59c
120	0e	0e	0e	0c

Table 3: Effect of 2,4-D in combination with 6-BA on callus induction of anthers

		Callus formation rate (%)			
2,4-Dconcentration (mg/L)	6-BA concentration (mg/L)	Bixiu	Dabai	Changbai	Pangniu
0.1	0.5	5.94f	3.25f	5.09f	8.49d
0.1	1.0	11.37e	9.79e	11.53e	10.04d
0.1	2.0	12.72e	10.08e	11.90e	9.75d
0.1	4.0	8.09e	11.32e	12.33e	11.63d
0.5	0.5	40.28c	29.64cd	39.82c	40.44b
0.5	1.0	59.71b	37.23c	41.47c	44.49b
0.5	2.0	79.42a	71.52a	73.14a	75.23a
0.5	4.0	56.37b	55.28b	59.12b	54.92b
1.0	0.5	26.58d	27.44d	24.50d	30.00c
1.0	1.0	35.72c	29.27cd	27.82d	31.19c
1.0	2.0	42.49bc	30.72cd	32.05cd	37.25bc
1.0	4.0	36.18c	30.51cd	30.32cd	27.92c

Table 4: Effect of sucrose concentration on callus induction of anthers

		Callus formation rate (%)			
Sucrose (%)	Callusing time (day)	Bixiu	Dabai	Changbai	Pangniu
0	\	0c	0c	0c	0c
3	5-7	88.27a	84.96a	86.88a	83.74a
5	7-14	76.61b	72.38b	73.92b	71.83b
7	7-14	40.22c	37.36c	36.21c	36.89c

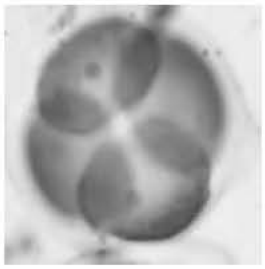

Fig. 1: Tetrad microspore

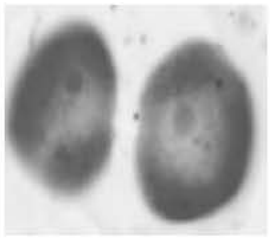

Fig. 2: Uninucleate microspore

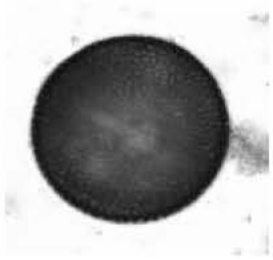

Fig. 3: Mature microspore

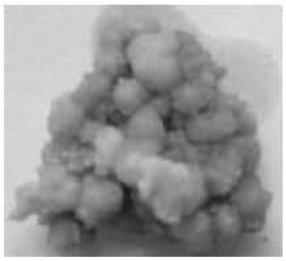

Fig 4: Browning necrosis callus

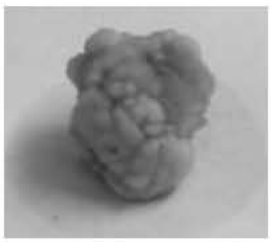

Fig 5: Green healthy callus

Fig 6: Viridescence vital callus

Effect of 2,4-D and BA on the Callus Formation from Anthers: The callus formation rate of balsam pears reached the highest when medium added with 2,4-D 0.5mg/ L and BA 2.0 mg/L and Bixiu reached 79.42% (Table 3). The callus formation rate was increased with the increasing of 2,4-D concentration from 0.1 mg/L up to 0.5 mg/L. However, with the concentration of 2,4-D up to 1.0 mg/L, the callus was prone to brown (Fig. 4). It was beneficial to inducing callus when the concentration of BA was 2.0 mg/L and the callus was green and vital (Fig.5& 6). While the concentration of BA was more or less than 2.0 mg/L, callus formation rate would be lower.

Effect of Sucrose on the Callus Formation from Anthers: Anthers cultured on the medium without sucrose were not formed callus (Table 4). When medium added with 3% sucrose, callus was derived from anther and anther wall, so the inductivity of callus was maximum. With the increasing of sucrose up to 5%, anther wall were restrained by sucrose and callus were derived from anther only, so inductivity of callus declined. Anthers cultured on the medium added with 7% sucrose were restrained by sucrose as well as anther wall and formed the least callus.

DISCUSSION

It was widely supposed that microspore development stage had a great effect on anther culture. For most plants, it was easier to form embryo or callus for pollen in late-uninucleate stage [4,5,6]. The results also showed that anthers of late-uninucleate stage were induced the most callus for balsam pear.

Application of cold pretreatment has become an essential measure to increase the efficiency of androgenesis in many species [7,8,9]. According to this experiment, callus induction rate of cv. Bixiu, Dabai and Pangniu increased after cold pretreatment while cv. Changbai showed a significant decline. Chen H. got a similar result in the callus formation from loquat anther [10]. However, the callusing potential of these four cultivars of balsam pear anthers was impaired by prolonged cold pretreatment. When the cold pretreatment was up to 120 h, no anthers formed callus and the anthers became necrotic within one week. Therefore, cold pretreatment was one of key factors for callus induction in balsam pear anther culture. And the definite effect on different cultivars of balsam pear need further study.

The presence of an appropriate concentration of growth regulators in the medium plays an important role on callus formation in anther culture. Especially 2,4-D was indispensable and critical for the dedifferentiation of anthers [11]. In the present study, anthers cultured on the medium supplemented with 2,4-D 0.5 mg/L and BA 2.0 mg/L resulted in the highest callus formation rate, which was similar with the result obtained in cucumber [12,13].

The results revealed that medium without sucrose was invalid for callus induction, which suggested that sucrose might be absolutely necessary for the dedifferentiation of balsam pear anthers. The results of earlier experiments showed that the callusing potential of anther wall and filament was impaired by high concentration of sucrose [14], which was proved in this experiment. Our study showed that the best callus formation rate from balsam pear anther was 5% sucrose.

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