Short Communication

Effect of different pretreatment on callus formation from anther in balsam pear (Momordica charantia L.)

Yi Tang, Xiaomei Li, Juan Li, Chao Ma, Jia Lai and Huanxiu Li*

College of Horticulture, Sichuan Agricultural University, Ya’an 62504, Sichuan, China.

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The effect of different pretreatment on callus induction from anther in three cultivars of balsam pear (Momordica charantia L.) was investigated. The results showed that cold pretreatment was the best for anther callus induction, and followed by heat shock, while centrifugation had no significant effect on callus induction from anther. Cold pretreatment for 24 h proved to be optimal as it significantly increased the callus induction rate, which varied with cultivars, reaching the maximum of 44.00, 51.10 and 72.67% for cv. Lvcui, Cuifei and Bixiu, respectively.

Key words: Balsam pear (Momordica charantia L.), anther culture, callus induction, pretreatment.

INTRODUCTION

Balsam pear (Momordica charantia L.) is an annual tendril herbage plant of gourd family. It contains high concentrations of ascorbic acid and iron (Behera, 2008), and it is popular with people as an important and valuable vegetable. Balsam pear has been used as a traditional medicine for diabetes in India, China, and Central America (Grover et al., 2002; Yeh et al., 2003). The traditional breeding methods are time-consuming and limited by space required for field experiments. Anther culture is an efficient method for plant breeding and can shorten breeding period (Andrea et al., 2001). It has become one of the major techniques in plant breeding programs (Bajaj, 1983). Compared with conventional inbreeding, the in vitro androgenesis technique enables a faster generation of virtually fully homozygous lines (Aulinger et al., 2003). Until now, there were few reports on anther culture in bitter melon and regeneration system of anther culture haven’t established. There are a number of factors that affect androgenesis, including genotype and physiological state of the donor plant, pollen developmental stage, pretreatment, physical factors and chemical factors (Sopory and Munshi, 1996). It is widely supposed that pretreatments had a great effect on anther culture. Our objective is to investigate the effects of different pretreatments on callus induction in balsam pear cv. Lvcui, Cuifei and Bixiu, to establish a high frequency anther culture system.

MATERIALS AND METHODS

Young viridescence flower buds about 5-mm-long in diameter were collected from balsam pear cv. Lvcui, Cuifei and Bixiu, which contained approximately 80% uninucleate microspores confirmed by periodically microscopically checking the microspore stage. The donor plants were grown in the experimental plots using standard agronomic practices. The flower buds were collected in a conical flask with a piece of wet cotton to do various pretreatments. 1. Three different pretreatments: hot shock (35° C, 6 h), cold pretreatment (4° C, 24 h), centrifugation (2000 r/min, 12 h), and with no any pretreatment as control (CK); 2. Different time last of cold pretreatment: under 4 ℃ condition for 0 (CK), 24, 48, 72, 96 and 120 h. Culturing operations of anthers were carried out in a laminar air-flow cabinet under aseptic conditions. The flower buds were dipped in 75% (v/v) alcohol for 30 s, immersed in 0.1% (w/v) mercuric chloride solution with periodic agitation for 5 min, and washed with sterile distilled water for five times. After filament removed, the intact anthers were inoculated on inducing medium. Callus induction media consisted of MS mineral salts and vitamins (Murashige and Skoog, 1962), 5% (w/v) sucrose, 0.6% (w/v) agar, supplemented with various types and concentrations of plant growth regulators including 2,4-dichlorophenoxyacetic acid (2,4-D), benzyladenine (6-BA). The media were adjusted to pH 5.8 prior to addition of agar and sterilized at 122 ℃ and 104 kPa pressure for 20 min. The anthers were cultured in a culture chamber at 25 ℃ in the dark for 10 days, and then at 25 ℃ under 16 h daily illumination with 1500 lx fluorescent light. A randomized complete block design was used for callus induction experiments. Each treatment was

*Corresponding author. E-mail: hxli62@163.com.

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; 6-BA, 6-benzyladenine; MS, Murashige and Skoog (1962).
Figure 1. Growth chart of callus formation from anther culture in balsam pear. A: An individual anther whose filament was completely removed, B: anther swelling after culture for 1 week, C: anther forming a large number of callus after culture for 4 week.

Table 1. Effect of pretreatments on callus induction from anthers in balsam pear.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Lvcui</th>
<th>Cuifei</th>
<th>Bixiu</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>25.33c</td>
<td>14.67c</td>
<td>38.33c</td>
</tr>
<tr>
<td>hot shock</td>
<td>36.33b</td>
<td>29.67b</td>
<td>53.00b</td>
</tr>
<tr>
<td>centrifugation</td>
<td>25.67c</td>
<td>18.67c</td>
<td>41.33c</td>
</tr>
<tr>
<td>cold pretreatment</td>
<td>46.67a</td>
<td>45.33a</td>
<td>70.33a</td>
</tr>
</tbody>
</table>

Means having the same letter in the columns were not significantly different according to Duncan’s multiple range test at P=0.05.

applied to 300 anthers (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 (Duncan, 1955).

RESULTS

Effect of pretreatments on the callus formation from anthers

The growth chart of callus formation from cultured anther in balsam pear was showed in Figure 1. Analysis of variance of the callus induction rate indicated that callus formation was significantly affected by pretreatments. According to the data in Table 1, cold pretreatment (4℃) is the best for anther callus induction in all the pretreatments, and followed by heat shock. No statistically significant difference was found in centrifugation and CK, while both were comparatively low. And the effects of pretreatments on callus induction rate changed with cultivars.

Effect of cold pretreatment time on the callus formation from anthers

The results of different cold pretreatment time on callus induction from anthers were summarized in Table 2. Callus formation rate of cv. Lvcui, Cuifei and Bixiu increased after anthers pretreated under 4℃ condition for 24 to 48 h. Prolonged cold pretreatment (96 to 120 h) dramatically decreased the potential of anthers to produce calluses, while it increased the frequency of anthers to turn brown and then become necrotic. When the cold pretreatment was up to 120 h, no anthers formed callus, and the anthers became necrotic within one week. 24 h cold pretreatment was beneficial as it significantly increased the induction rate of callus, reaching the maximum of 44.00, 51.10 and 72.67% for cv. Lvcui, Cuifei and Bixiu, respectively.

DISCUSSION

It is widely supposed that pretreatment plays a key role for anther callus induction. The main pretreatments applied to anther culture are cold pretreatment, centrifugation, hot shock, water culture, and so on (Sangwan-Norreel, 1977; Wilson et al., 1978; Keller, et al., 1983). The effects of different pretreatments, including hot shock (35℃, 6 h), cold pretreatment (4℃, 48 h) and centrifugation (2000 r/min, 12h) on callus induction from anther were investigated in this experiment. The results showed that cold pretreatment is the best for anther callus induction, and followed by heat shock, while centrifugation had no effect on anther callus induction. Application of cold pretreatment has become an essential measure to increase the efficiency of androgenesis in
Table 2. Effect of cold pretreatment time on callus induction from anthers in balsam pear.

<table>
<thead>
<tr>
<th>Cold pretreatment time (h)</th>
<th>Lvcui Callus formation rate (%)</th>
<th>Cuifei</th>
<th>Bixiu</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>22.33b</td>
<td>17.00c</td>
<td>39.33c</td>
</tr>
<tr>
<td>24</td>
<td>51.10a</td>
<td>44.00a</td>
<td>72.67a</td>
</tr>
<tr>
<td>48</td>
<td>47.33a</td>
<td>40.33a</td>
<td>60.33b</td>
</tr>
<tr>
<td>72</td>
<td>27.67b</td>
<td>29.33b</td>
<td>37.00c</td>
</tr>
<tr>
<td>96</td>
<td>10.33c</td>
<td>7.67d</td>
<td>16.67d</td>
</tr>
<tr>
<td>120</td>
<td>0d</td>
<td>0e</td>
<td>0e</td>
</tr>
</tbody>
</table>

Means having the same letter in the columns were not significantly different according to Duncan’s multiple range test at P=0.05.

many species (Pechan and Smykal, 2001). In our study, we found that the cold pretreatment of flower buds was necessary for callus induction from anthers in balsam pear, and cold pretreatment for 24h proved to be optimal as it significantly increased the induction rate of callus. However, the callusing potential of these three 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.

The results of our research revealed that the callus induction was also influenced by cultivars. In this study, balsam pear genotype has a significant effect on callus induction; callus induction rate of cv. Bixiu is highest, followed by cv. Lvcui and cv. Cuifei. Many studies have shown that the genotype of donor plants plays an important role in anther culture (Chen et al., 2007). Kiefer et al. (1993) reported anther culture of kale (Brassica oleracea) determined by genotypes, and Lu et al. (1991) found callus induction of different genotypes of Brassica varied between 0 and 35%. Therefore, we should select the genotype which can produce high frequency of callus induction firstly in anther culture of balsam pear.

ACKNOWLEDGEMENTS

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REFERENCES


