Detection of cucumber parthenogenic haploid embryos by floating the immature seeds in liquid medium

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Abstract
Only a small percentage of seeds arisen by irradiated pollen include haploid embryo. Inspecting the seeds one by one for rescuing the embryos is a tedious and time consuming work which decreases haploid production efficiency in this method for cucurbit plants. Cucumber seeds formed three weeks after pollination with irradiated pollen were floated in liquid medium for about 10 days before excision of the embryos. This innovative method allowed a better development of haploid embryos; they turned to green and germinate like normal seeds and could be identified easily without the need of inspecting the seeds one by one. This method permitted an easier detection and extraction of embryos in comparison with common approach, increased the number and the quality of rescued embryos and finally increased substantially the percentage of recovered haploid plants. This method was already mentioned for melon and we demonstrated its interest in cucumber. In addition, the comparison of different conditions for preparing and culturing seeds in liquid medium, indicated that washing the seeds in running water and then sterilizing them with commercial bleach for ten minutes was more efficient than opening the fruits and extracting the seeds under laminar air flow.

INTRODUCTION
Doubled haploids (DH) have contributed to breeding programs for diverse crops. Obtaining melon haploids achieved by rescue of parthenogenetic embryos induced by pollination with irradiated pollen (Sauton and Dumas de Vaulx 1987) was the first reported success in cucurbit crops. This was confirmed and investigated further by other authors working with melon (Cuny et al. 1992; Dore et al. 1995; Ficcadenti et al. 1995), cucumber (Sauton 1989; Caglar and Abak 1999; Lotfi et al. 1999), and watermelon (Sari et al. 1994). Use of the technique in applied breeding programs has been limited by the low percentage of haploid embryos induced, the difficulty in detecting and excising these embryos, and the small numbers of haploid plantlets recovered. Savin et al. (1988) and Sauton et al. (1989) suggested the use of soft X-ray technique to detect embryos within the seeds, but the equipment required is not available in many laboratories and it’s not efficient in practice (Claveria et al. 2005). Lotfi et al. (2003) used an easy and improved procedure for detecting embryos in melon. They put seeds in liquid culture before excision of the embryos, so haploid
embryos developed more easily and were identified without the need to inspect the seeds one by one. In this paper we describe the method of floating immature seeds in liquid medium for detecting cucumber parthenogenic haploid embryos induced by irradiated pollen.

MATERIAL AND METHODS

Two commercial F₁ hybrids (‘Soltan’ and ‘Monarch’) and four local cultivar of cucumber were used as the maternal plants. The plants were grown in greenhouse. Male flowers were collected the day before anthesis and irradiated with 250 Gray gamma rays. On the following day, female flowers were pollinated with the irradiated pollen. Fruits were harvested 21-23 days after pollination and seeds were removed either aseptically in a laminar flow hood or under running water in the laboratory, sterilized for 10 minutes with 20 % Clorox and rinsed with sterile water. Seeds were then placed unopened in E20A liquid medium (Sauton and Dumas de Vaulx 1987) in 100 x 20 mm Petri dishes (about 40 seeds per dish). Seeds were cultured at 25°C with a 16h light: 8 h dark photoperiod (~50 µmol/m²/sec) with occasional shaking by hand (Fig. 1). After about 10 days of culture, seeds were examined over a light box containing a small white fluorescent lamp. Seeds that appeared to contain embryos were opened aseptically. Embryos were excised and transferred to plates (3 embryos per plate) of solid E20A medium and cultured for approximately 2-3 weeks. Embryos that grew well on plates were transferred to E20A solid medium in baby food jars with transparent caps. Subsequently each plantlet was propagated by putting the shoot tip and single stem nodes into fresh medium and rooted plants were transplanted to soil, acclimatized and were transferred to the greenhouse.

RESULTS AND DISCUSSION

Three weeks after pollination with irradiated pollen, only a small percentage of the seeds contained embryos. A total of 48 parthenogenetic embryos were recovered giving 25 plants. The seeds that contained embryos were easily detected over a light source. Some of these embryos had turned green within the seeds, and 3 embryos actually germinated like normal seeds (Fig. 1). In addition, excision of embryos from seeds grown in liquid medium caused less injury to the embryos. More than 400 seeds could be inspected and viable embryos rescued within one hour. In contrast, opening all the seeds within a fruit took several hours of careful and tedious work. The values in Table 1 for this treatment are comparable with those in previous work (Sauton

Figure 1. From left to right: seeds cultured in liquid medium in growth chamber, germinated embryo in liquid medium, rescued embryos in solid medium

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1989; Przyborowski 1994; Caglar and Abak 1999; Lotfi et al. 1999) although precise comparisons are difficult because of differences in genotypes, seasonal effects, and data provided. Almost half of the embryos excised after seed culture were at a more developed stages from which more than 80% plant recovery was done for all genotypes. This confirms the benefits of the liquid seed culture method (Lotfi et al. 2003). It was necessary to open less than 100 from 18,774 (total number of non-contaminated) seeds to obtain enough embryos to recover 25 plants whereas all of them should be opened for inspecting directly.

Some Petri dishes of seeds cultured in liquid medium were contaminated. The mean percentage of seeds lost to contamination was about 22%. Contamination was lower (7.8%) for seeds that were Chlorox sterilized than for those removed aseptically from sterilized fruits (35.6%). The two procedures for handling the embryos gave markedly different results (Tab. 2). Contamination problem was more severe in the liquid culture method because a single contaminated seed could result in loss of the entire Petri dish. In comparison with the advantages of liquid culture detailed above, this problem was not severe and could be minimized by more stringent sterilization and by culture of fewer seeds per plate. For future work, sterilization of seeds rather than fruits is recommended. An additional benefit of this approach is that it conveniently removes placental and fruit tissues. It would also be advisable to put the Petri dishes on a shaker at low speed (30 rpm) for better aeration.

Table 1. Parthenogenetic embryos and plants recovered after floating of seeds in liquid culture for different cucumber genotypes.

<table>
<thead>
<tr>
<th>Cucumber genotype</th>
<th>Number of seeds</th>
<th>Number of embryos</th>
<th>Percentage of embryos per seed</th>
<th>Globular or heart shaped embryo</th>
<th>Developed embryos</th>
<th>Abnormal embryos</th>
<th>Total number of plants</th>
<th>Percentage of plants per embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Soltan’</td>
<td>5345</td>
<td>21</td>
<td>0.39</td>
<td>5/1</td>
<td>15/12</td>
<td>1/0</td>
<td>13</td>
<td>65</td>
</tr>
<tr>
<td>‘Monarch’</td>
<td>3762</td>
<td>12</td>
<td>0.32</td>
<td>5/2</td>
<td>7/3</td>
<td>0</td>
<td>5</td>
<td>42</td>
</tr>
<tr>
<td>G5</td>
<td>2435</td>
<td>6</td>
<td>0.24</td>
<td>2/0</td>
<td>3/3</td>
<td>1/0</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>G46</td>
<td>970</td>
<td>1</td>
<td>0.10</td>
<td>1/1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>G66</td>
<td>3167</td>
<td>3</td>
<td>0.09</td>
<td>2/0</td>
<td>1/1</td>
<td>0</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>G70</td>
<td>3095</td>
<td>6</td>
<td>0.19</td>
<td>2/1</td>
<td>3/1</td>
<td>1/0</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>18774</td>
<td>49</td>
<td>0.26</td>
<td>17/5</td>
<td>29/20</td>
<td>3/0</td>
<td>25</td>
<td>51</td>
</tr>
</tbody>
</table>

x Number of globular or heart shaped embryos / Number of plants derived from these embryos
y Number of green or rooted embryos / Number of plants derived from these embryos
z Number of abnormal or torpedo stage embryos / Number of plants derived from these embryos

While the efficiency of this method has been shown before in melon and was emphasized in our work for cucumber, the same procedure is likely to be effective and beneficial with other plants which are derived through parthenogenesis and especially beneficial for those standard recovery of plants is low.
Table 2. Comparison of different conditions for preparing and culturing seeds in liquid medium: A: opening fruits and washing seeds under laminar air flow. B: washing seeds in sink and then sterilization with Chlorox for 10 min.

<table>
<thead>
<tr>
<th>Condition of preparing seeds</th>
<th>Total number of seeds</th>
<th>Number of contaminated seeds</th>
<th>Percent of contamination</th>
<th>No. of rescued embryos</th>
<th>Percent of rescued embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Under laminar</td>
<td>14875</td>
<td>5295</td>
<td>35.6</td>
<td>17</td>
<td>0.17</td>
</tr>
<tr>
<td>B: Chlorox sterilizing</td>
<td>9972</td>
<td>778</td>
<td>7.8</td>
<td>31</td>
<td>0.34</td>
</tr>
<tr>
<td>Sum or mean</td>
<td>24847</td>
<td>6073</td>
<td>21.7</td>
<td>48</td>
<td>0.25</td>
</tr>
</tbody>
</table>

ACKNOWLEDGMENT
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Literature cited


