Comparative evaluation of different embryo rescue techniques on parthenogenetic melon (Cucumis melo L.) fruits induced with irradiated pollen

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The methods of dihaploidization ensures important advantages in obtaining pure lines by rapid fixation of homozygosity. "Irradiated pollen technique" was used in this study. Disease resistant F1 hybrid variety candidates were utilized as the plant material. For the parthenogenetic induction, 300 Gray gamma irradiation were applied to the pollens and pollinations were conducted on the day after irradiation. Three different methods were used to extract seeds from the 3 to 4 weeks old fruits. The three methods: extracting the seeds one by one (M1), sowing the seeds in petri dishes (M2) and inspecting the seeds on the light box (M3) were compared. The pollination with the irradiated pollens resulted up to 94% fruit set. From the 204 fruits harvested, 280 haploid, 44 diploid and 8 mixoploid embryos were obtained. Germination rate of haploid was 96%. When the M1, M2 and M3 were compared, the required time for opening a fruit was found to be 162, 125 and 49 min, respectively. The M3 method was identified as the most successful, economic and effective. The results are expected to lower the cost and increase the efficiency of dihaploidization use in melon breeding.

Key words: Melon, Cucumis melo L., haploid, dihaploidization, irradiated pollen, embryo rescue.

INTRODUCTION

Doubled haploidy is the fastest way to reach homozygosity. The use of dihaploidization especially in the studies of quantitative resistance offers the breeders and geneticists unique advantages since the inbreeding process of particularly outcrossed species requires two folds time owing to the backcrosses.

Haploids and doubled haploids can be obtained via the routes of androgenesis, gynogenesis or pollination with incomplete pollen (treated with chemicals, irradiated etc). It is possible to benefit from more than one route in potato and some monocotyledonous species. Conversely, the possible choice is generally restricted by only one route in most dicots.

According to Guis et al. (1998), using androgenesis and gynogenesis has not been successful in producing haploid melon plants (Nunez-Palenius et al., 2009).

Pollen irradiation with Co$^{60}$γ-rays is the most widely used technique to induce in situ gynogenetic haploid parthenogenesis in melon at the present. This technique consists of the induction of in situ gynogenic embryos with gamma-ray irradiated pollen and then followed by rescue of haploid embryos by in vitro culture. In this technique, irradiated pollen germinates on the stigma, but it cannot fertilize the egg-cell and the polar nuclei. Pollen induces the division of the egg cell and thus induces parthenogenesis and gynogenic haploid embryo (Kurtar et al., 2009).

The first successful dihaploidization study (irradiated pollen technique) on melon was performed by Sauton and Dumax de Vaulx (1987), followed by Brun (1990), Cuny et al. (1992), Maestro-Tejada (1992), Sari et al. (1992), Abak et al. (1996), Yanmaz and Taner (1996), Sari et al. (1999), Bal et al. (2003), Yetisir and Sari (2003), Taner et al. (2003), Lotfi et al. (2003), YongBing et al. (2007), Lim and Earle (2008), Lotfi and Salahia (2008), Berber (2009), Kasapoglu (2009), Gursoy (2009) and Baktemur (2009). However, it should not be ignored...
that various factors such as genotype, environmental elements, embryo yield and irradiation dose have extremely large effect on the success of irradiated pollen technique (Sari et al. 1992; Ficcadenti et al. 1995; Doré et al. 1995). Furthermore, the dependence on a radiation source and the stage of embryo rescue are the two most important factors limiting the practical use of this technique.

According to the literature, the most referenced method in the embryo rescue stage has been to extract seeds one by one with hand. This method requires long time and qualified labor and restricts its fast and effective usage in the commercial or scientific studies in which intensive breeding programs are carried out. However, for shortening the embryo rescue stage of irradiated pollen technique, some studies (Savin et al., 1988; Sauton et al., 1989; Lotfi et al., 2003; Claveria et al., 2005; Lotfi and Salah, 2008; Lim and Earle, 2008; Baktemur, 2009) were conducted. With the same purpose, the present study was carried out. In this study, three different embryo rescue methods were compared in terms of the time spent and the cost within a whole frame of irradiated pollen technique of which all stages were completed till acclimatization.

**MATERIALS AND METHODS**

**Plant material**

This study was conducted in Bati Akdeniz Agricultural Research Institute (BATEM), Antalya, Turkey in 2006. Plant material was obtained from the melon breeding program of BATEM. After testing F1 melon variety candidates against the races (0, 1, 2, and 1-2) of *Fusarium oxysporum* f.sp. *melonis*, the candidates with resistance to all four races were used as the maternal parent. Mixed pollens collected from 10 different genotypes of the breeding program were utilized as pollen source. The seeds were sown in plastic pots with dimensions of 8 x 8 x 8 cm with a mixture of 1:1 peat and perlite medium on January 30, 2006. Seedlings were planted in unheated glasshouse on 03.03.2006. Seedlings were transplanted according to double line methods with (1 x 0.5) x 0.5 m spacing. Plants were grown with a single stem by pruning and hanging. Plant nutrition compounds were supplied by drip-irrigation system on the basis of soil analyses and plants were protected with insecticides and fungicides regularly throughout the growing season.

**Parthenogenetic induction with irradiated pollen**

The fruits obtained through parthenogenetic induction were based on the method of Sari et al. (1999). The stages of the method were as follows:

**Irradiations**

Male flowers of the plants were collected a day before anthesis. After removing from sepal and partially petal leaves, they were placed in petri dishes. They were irradiated with 300 Gy gamma rays in a Coγ reactor at three different times (May 2, 12 and 16) (Table 1 and Figure 1). The first irradiation was made at Turkish Atomic Energy Authority in Ankara, which is 500 km away from Antalya, transporting the pollen via airplane on the same day. The second and third irradiations were conducted at Akdeniz University Medical School, Department of Radiation Oncology in Antalya.

**Emasculations**

On the very same days when the male flowers were collected for irradiation, female flowers were emasculated (Table 2 and Figure 1). After emasculations, the flowers were isolated with cellophane pouches against pollen contaminations.

**Pollination**

Emasculated female flowers were pollinated with the irradiated pollens between 5.50 and 9.30 am on the following day of irradiations (Table 3 and Figure 1).

After the first irradiation, half of the irradiated flowers were treated with n-pentane (CH$_3$(CH$_2$)$_3$CH$_3$) (MERCK) solution in order to keep irradiated pollens for use on the following day. For this application, with the help of a forcep, the flowers were dipped into the solution in a 6 cm-diameter-petri dish. After evaporation of volatile n-pentane solution, dried pollens were kept at 4°C and used for pollination the next day. Thus, the first pollination application lasted for 2 days (May 3 and 4). Images of the stages of parthenogenetic induction are shown in Figure 1.

**In vitro studies**

**Disinfection**

The 3 - 4 weeks old unripen melon fruits obtained from pollinations with irradiated pollens were harvested. They were washed under tap water and treated with 25% sodium hypochloride solution for 20 min. After rinsing and drying, the fruits were disinfected via dry burning method with 96% ethanol on fire in a laminar flow hood (Figure 2).

**Embryo rescue**

The fruits were cut transversally after disinfection. Then, the seeds were removed aseptically and put into the 9 cm-diameter-petri dishes. Three different methods, namely: M1, Extracting the seeds one by one; M2, sowing the seeds in petri dishes; M3, inspecting the seeds under the light, were tested for the determination of the seeds with haploid embryo. With regard to haploid embryogenesis stages, only heart-shaped embryos were taken into consideration,
Figure 1. The stages of parthenogenetic melon fruit induction. A1 - A3, preparation of male flowers for gamma irradiation, B1 - B3, emasculation processes; C1 - C3, pollination processes.

Table 2. Emasculations of the flowers on the mother plants conducted one day before anthesis.

<table>
<thead>
<tr>
<th>Emasculations</th>
<th>Date of emasculation</th>
<th>The number of emasculated female flower</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Emasculation</td>
<td>May 2, 2006</td>
<td>68</td>
</tr>
<tr>
<td>II. Emasculation</td>
<td>May 12, 2006</td>
<td>128</td>
</tr>
<tr>
<td>III. Emasculation</td>
<td>May 16, 2006</td>
<td>118</td>
</tr>
</tbody>
</table>
Table 3. Pollination dates.

<table>
<thead>
<tr>
<th>Pollinations</th>
<th>Date of pollination</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Pollination</td>
<td>May 3 - 4, 2006</td>
</tr>
<tr>
<td>II. Pollination</td>
<td>May 13, 2006</td>
</tr>
<tr>
<td>III. Pollination</td>
<td>May 17, 2006</td>
</tr>
</tbody>
</table>

Figure 2. Rescue and culture of haploid embryos. A1 – A2, Disinfection of the fruits via dry burning method; B1 - B2, method 1: embryo rescue by extracting seeds one by one; C1 - C2, method 2: embryo rescue by sowing seeds in petri dishes; D1 - D2, method 3: embryo rescue by inspecting seeds on the light; E1 - E3, germination of haploid embryos and transferring them to the culture tubes.
while the globular-shaped embryos or the others were not evaluated in the study. The number of diploid and mixoploid embryos was also counted beside haploid ones.

The three pollinations with irradiated pollens resulted to 246 melon fruits, 204 of which were used to compare the three embryo rescue methods. The process of opening the fruits, extraction of the seeds and rescue of haploid embryos were carried out between May 23 and June 21, 2006.

**Extracting the seeds one by one:** All the seeds extracted from the fruits were individually opened by hand with the help of forceps and lenset one by one under binocular microscope (OLYMPUS SZ251). The extracted haploid embryos were directly transferred to the germination medium. With this method, 1 skilled person working for 7 h in a day opened 13 fruits in 5 days between May 23 and June 6 (1 person/13 fruits/35 hour/5 days).

**Sowing the seeds in petri dishes:** All the seeds extracted from the fruits were placed side by side onto solid MS (Murashige and Skoog, 1962) medium containing 0.01 mg/l indole acetic acid (IAA) in 9 cm diameter petri dishes. They were incubated at 25°C in dark. After 4 - 5 days incubation, the petri dishes were examined over a light box containing a small white fluorescent lamp. The location of the seeds appearing to contain haploid embryos was marked. Then, they were opened aseptically and embryos were transferred to the germination medium.

For this method, 25 L tissue culture medium and 550 petri dishes were used for sowing of the seeds. 2 skilled person, each working for 7 h a day, opened 87 fruits in 13 days between May 24 and June 14 with this method (2 person/87 fruits/182 hour/13 days).

**Inspecting the seeds on the light box:** All the seeds extracted from the fruits were placed aseptically side by side onto a light box containing a small white fluorescent lamp in the laminar flow hood. The seeds that seemed to contain haploid embryos were opened immediately and embryos were transferred to the germination medium. 2 skilled person, each working for 7 h in a day, opened 104 fruits in 6 days between June 15 and 21 with this method (2 person/104 fruits/84 h/6 days). Images of the methods used for seed and embryo extraction are given in Figure 2.

**Germination of embryos**

All the haploid embryos extracted from the seeds were sown on the germination medium composed of solid E20A (Sauton, 1987) medium and 0.01 mg / l IAA in small jars approximately 4 cm diameter and height. The embryos in jars were cultured at 25°C and 16 h light: 8 h dark photoperiod (the growth room conditions are the same for all in vitro cultures henceforth). After the initiation of germination, the embryos 2 - 3 cm in size with roots and shoots were transferred to the culture tubes 3 cm in diameter and 20 cm in height (Figure 2).

**Transfer to shoot multiplication medium**

Some of the genotypes transferred to culture tubes showed unusual development in the form of abnormal hypocotyl elongation due to insufficient light intensity in the growth room. Since this growth would not be suitable for the subsequent cloning stage, 57 haploid and 12 diploid genotypes were transferred to a shoot multiplication medium composed of E20A medium + 0.1 mg/l IAA (indole acetic acid) + 0.5 g/l BAP (benzyl amino purine) in June 28. The diploid embryos were used as control. Thus, it was possible to compare the growth of haploid embryos with diploid one’s in all stages beginning from in vitro culture to in vitro colchicine treatment.

**Cloning**

The cloning process was required to multiply haploid genotypes consisting of only one copy against the risk of probable losses after the subsequent stages of colchicine treatment and acclimatization. For this purpose, a total of 165 genotypes (151 haploid and 14 diploid) was cloned. The clones were transferred to a medium consisting of E20A medium + 0.1 mg/l IAA. Additionally, the lower parts (below the shoots) of the 94 genotypes, cut just before colchicine treatment, were transferred to the same fresh nutrient medium for conservation as the backup clones.

**Acclimatization**

The in vitro plantlets about 15 - 20 cm in height obtained from the study were subjected to acclimatization process. This process was initiated as plantlets were grown under in vitro conditions. At the outset, cover of the culture tubes was opened gradually for 5 - 6 days. Then, plantlets were removed from the tubes. Plantlets were washed under tap water to remove the excess agar in the roots. Subsequently, the roots were dipped in a solution of “BENLATE” and “CAPTAN” mixture (0.25% v/v) for 10 min to prevent a possible contamination in the course of acclimatization. Afterwards, the seedlings were transplanted into plastic cups (120 cm³) containing sterilized peat moss. Each cup was covered with a transparent plastic bag and the plants were acclimatized in a growth chamber with approximately 94% relative humidity. The transparent bags were gradually opened and removed entirely within 6 days. Humidity of the growth chamber was periodically reduced at intervals of 5% in each 2 days until humidity attained greenhouse conditions.

**RESULTS**

**Pollination and fruit sets**

The rates of fruit sets obtained from pollination are given in Table 4. The highest fruit set was achieved from the second pollination with 94.5%, followed by the third one with 86.4%, and the first pollination with average of 33.8%. In the first pollination, 54.5% fruit set occurred with irradiated pollen where pollination was made on May 3rd, while n-pentane treated irradiated pollen used on May 4th resulted to a 14.2% fruit set. Thus, average fruit set rate of all three pollinations was found to be 71.6%.

**Embryos**

Of the 246 fruits obtained from pollinations, 204 of them were opened. The haploid embryos were rescued by one of the three different rescue methods (Methods 1, 2 or 3).
The results are summarized in Table 5. A total of 332 embryos (280 haploid, 44 diploid and 8 mixoploid) was acquired (Table 5). Of the 23 fruits obtained from the first pollination, 5 haploid embryos were recovered. The average number of haploid embryo per fruit was found to be 0.22. In the second pollination, from the 115 fruits, 165 haploid embryos were acquired. The average number of haploid embryo per fruit was found to be 1.43. From the 66 fruits that originated from the third pollination, 110 haploid embryos were obtained. The average number of haploid embryo per fruit was found to be 1.67. Thus, the mean number of haploid embryo per fruit was determined to be 1.37 in the study.

The number of haploid embryo rescued from the fruits ranged from 0 to 20. The highest haploid embryo (20 pieces) was obtained from the fruit number 167 acquired from the third pollination and rescued with M3. Of the 280 haploid embryos, 269 of them germinated. Thus, the rate of embryo germination was 96% in this study. For all the germinated embryos that were transformed into in vitro plantlets, the transformation rate of embryo to plant was 100%.

Embryo rescue methods
The data obtained from 3 different embryo rescue methods (M1, extracting the seeds one by one; M2, sowing the seeds in petri dishes; M3, inspecting the seeds on the light) are presented in Table 6. The mean number of fruit opened in a day (fruit/day) was 2.6, 6.7 and 17.3 for M1, M2 and M3, respectively. The efficiency of methods in terms of time spent per fruit ranged from 0.37 to 1.24 fruit/h; M3 being the most efficient and M1 being the least efficient method (Table 6). The mean time spent per fruit (minute/fruit) was found to be 162.0, 125.4 and 48.6 min, respectively. When the methods were compared with regard to cost per fruit opening, M3 was the cheapest (3.81 $) and M2 was the most expensive (40.62 $) (Table 7). The added cost of consumables (disposable petri, prepared media, sucrose, agar, IAA and others) increased the total and mean cost of M2. The cost of machinery equipment such as the binocular microscope used in M1 and the light box used in M2 and M3 were not included in the calculation of the total cost. The labour and other
Table 6: Comparison of different embryo rescue methods

<table>
<thead>
<tr>
<th>Embryo rescue method</th>
<th>Number of opened fruits</th>
<th>Day number of applied technique</th>
<th>Opened fruit number per day (fruit / day)</th>
<th>Opened fruit number per hour (fruit / hour)</th>
<th>Spent time per fruit (hour / fruit = minute / fruit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1* (extracting seeds one by one)</td>
<td>13</td>
<td>5</td>
<td>2.6</td>
<td>0.37</td>
<td>2.70 hour = 162.0 minute</td>
</tr>
<tr>
<td>M2** (sowing seeds in petri)</td>
<td>87</td>
<td>13</td>
<td>6.7</td>
<td>0.48</td>
<td>2.09 hour = 125.4 minute</td>
</tr>
<tr>
<td>M3** (inspecting seeds on the light)</td>
<td>104</td>
<td>6</td>
<td>17.3</td>
<td>1.24</td>
<td>0.81 hour = 48.6 minute</td>
</tr>
</tbody>
</table>

*: 1 person worked average 7 h per day for fruit opening (1 person / 7 h / day)
**: 2 person, each of whom spent average 7 hours in a day; worked totally 14 h per day for fruit opening (2 persons / 14 h / day)

Table 7. Comparison of different embryo rescue methods for cost.

<table>
<thead>
<tr>
<th>Embryo rescue method</th>
<th>Opened fruit number per day (fruit/day)</th>
<th>Required time (day / 100 fruit)</th>
<th>Labour cost*** ($ / 100 fruits)</th>
<th>Extra consumables cost **** ($ / 100 fruits)</th>
<th>Total cost ($ / 100 fruits)</th>
<th>Cost per fruit opening ($ / 1 fruit opening)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1* (extracting seeds one by one)</td>
<td>2.6</td>
<td>38.46</td>
<td>1269.18*</td>
<td>-</td>
<td>1269.18</td>
<td>12.70</td>
</tr>
<tr>
<td>M2** (sowing seeds in petri)</td>
<td>6.7</td>
<td>14.92</td>
<td>984.72**</td>
<td>3077.00****</td>
<td>4061.72</td>
<td>40.62</td>
</tr>
<tr>
<td>M3** (inspecting seeds on the light)</td>
<td>17.3</td>
<td>5.78</td>
<td>381.48**</td>
<td>-</td>
<td>381.48</td>
<td>3.81</td>
</tr>
</tbody>
</table>

*: 1 Person worked per day for fruit opening (1 person / day); ** 2 persons worked per day for fruit opening (2 persons / day); *** daily wage of a qualified worker was accepted to be $33; **** except for machinery equipment, cost such as binocular microscope or light box and their amortization shares; ***** total consumables cost including prepared in vitro culture media, sucrose, agar, IAA and disposable petri dishes used in M2 (sowing seeds in petri) was calculated to be $400 for 13 fruits, consequently $30.77 for each fruit.

In vitro colchicine treatment and cloning

The results of colchicine treatment are summarized in Table 8. For each genotype, 1 to 5 clones cut from the shoots right after colchicine treatment were obtained. After colchicine treatments, the formation of short node intervals and small leaves were observed as expected from doubled haploid genotypes. Along with 302 clones obtained soon after in vitro colchicine treatments, the total number of plantlets reached 610 clones in the study including all in vitro cultures performed for cloning of haploid genotypes, shoot multiplications and backup conservations.

Acclimatization

A total of 210 plantlets were subjected to acclimatization between July 28 and September 9 (Table 9). The 200 healthy plants subjected to acclimatization were transferred to a greenhouse into pots in September 9, 2006.

DISCUSSION

The mean percent fruit set ranged from 33.8 to 94.5 (average 71.6%) in our study. In similar parthenogenetic induction studies performed on different melon genotypes, Sauton and Dumas de Vaulx (1987) reported a 35% Maestro-Tejada
Table 8. *In vitro* colchicine treatments.

<table>
<thead>
<tr>
<th>Group of colchicine treatment</th>
<th>Date of treatment</th>
<th>Number of treated genotypes</th>
<th>Number of clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>July 21, 2006</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>II.</td>
<td>July 22, 2006</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>III.</td>
<td>July 24, 2006</td>
<td>22</td>
<td>70</td>
</tr>
<tr>
<td>IV.</td>
<td>July 25, 2006</td>
<td>41*</td>
<td>133</td>
</tr>
<tr>
<td>V.</td>
<td>July 26, 2006</td>
<td>17</td>
<td>111</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>94**</td>
<td>302</td>
</tr>
</tbody>
</table>

* 3 Genotypes were diploid; ** 91 genotypes were haploid.

Table 9. Acclimatization groups.

<table>
<thead>
<tr>
<th>Acclimatization groups</th>
<th>Operation date</th>
<th>Number of applied genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>July 28 – August 8, 2006</td>
<td>10</td>
</tr>
<tr>
<td>II</td>
<td>August 10 - 21, 2006</td>
<td>10</td>
</tr>
<tr>
<td>III</td>
<td>August 14 - 24, 2006</td>
<td>20</td>
</tr>
<tr>
<td>IV</td>
<td>August 22 - 31, 2006</td>
<td>80</td>
</tr>
<tr>
<td>V</td>
<td>August 24 - September 3, 2006</td>
<td>20</td>
</tr>
<tr>
<td>VI</td>
<td>August 31 – September 9, 2006</td>
<td>70</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>210</td>
</tr>
</tbody>
</table>

(1992), 35 to 87%, Sari et al. (1999), 25 to 54% (average 37%), YongBing et al. (2007), 50%, Gursoy (2009), 28.2 to 90.9% (average 58.5%) and Kasapoglu (2009) reported 28.7 - 76.2% (average 51.6%) fruit set rates in their studies. When compared to these studies, the highest periodic (94.5%) and average (71.6%) fruit set was achieved in our study and Kasapoglu (2009) reported 28.7-76.2% (average 51.6%) fruit set rates in their studies. When compared to these studies, the highest periodic (94.5%) and average (71.6%) fruit set was achieved in our study.

Gursoy (2009) and Kasapoglu (2009) reported that the highest fruit set was achieved from the pollinations made between May 4 and 11 in their studies. It was recommended that the seeding date should be adjusted according to the most suitable period for pollination to be performed between the end of April and May in melon haploidy studies. In fact, Gursoy (2009) and Kasapoglu (2009) succeeded in a 90.9 and 76.2% fruit set, respectively, from their pollinations made during this period. Similarly, the highest rate of fruit set (94.5%) of our study was also obtained from the same pollination period (May 12).

The environmental conditions of the petri dishes such as O<sub>2</sub> level, the rate of humidity and temperature value, in which the pollens were kept during transportation and irradiation process and waited until pollinations, can effect the viability of the pollens and consequently the success of fruit set. Indeed, suitable radiation dose has been known as the dose from which generative nucleus of the pollen can be damaged, but the pollen was not killed completely. Therefore, apart from the factor of appropriate pollination period, the reason of less fruit set of the first pollination in our study might have been caused by the transportation conditions. The first pollination was conducted with the pollens irradiated in Ankara, while the second and third ones were made with the pollens irradiated in Antalya. Briefly, without ignoring the significance of suitable pollination date; this result indicates the importance of irradiation center’s proximity to the breeding station.

In addition to successful parthenogenetic induction of the 280 haploid embryos, 269 were germinated and subsequently transformed to *in vitro* plantlets. Thus, 96% embryo germination and 100% transformation rate of embryo to plant were achieved in the study.

Although irradiated pollen technique has been a very valuable method for the breeders, the embryo rescue stage of the technique is the most troublesome step and restricts significantly the use of technique quickly and practically. However, some studies (Savin et al., 1988; Sauton et al., 1989; Claveria et al., 2005; Lotfi et al., 2008; Baktemur, 2009) were performed for abbreviation of the embryo rescue stage of this technique. Savin et al. (1988) and Sauton et al. (1989) pioneered on this issue for melon species. They suggested soft x-ray technology in order to detect haploid embryos in immature seeds. However, they reported that x-rays had been only efficient to discriminate 4-7 weeks old embryos in the
adequately desiccated seeds. Because of the deformations in the embryos starting after 3 - 4 weeks from the pollinations and nonexistence of the required machine in many laboratories, this technology did not find wide acceptance. Indeed, Claveria et al. (2005) reported that they had found this method ineffective in practice in their study on \textit{Cucumis sativus}. The studies carried out for shortening the duration of embryo recovery stage of irradiated pollen technique continued with Lotfi et al. (2003) and Lim and Earle (2008) studies on melon species. In both studies, the parthenogenetic seeds of melon were first cultured in a liquid medium for 10 - 15 days and later haploid embryos were detected by examining the seeds over a light box. Baktemur (2009) recently compared 5 different embryo rescue techniques in melon between 2007 and 2008. As for our study, three embryo rescue methods were compared. Our results are in agreement with Baktemur (2009). The M1 "extracting seeds one by one" required the maximum time for opening of a fruit (162 min = 2.7 h / 1 fruit), while the M3 "inspecting seeds on the light" took the shortest time (48.6 min = 0.81 h / 1 fruit) in our study. Similarly, the maximum time for opening of a fruit (103.5 min / fruit) was also found in the method of "extracting seeds one by one" in Baktemur (2009) as well. However, duration of this method is completely up to hand skills and experience of the working person. In this context, the M3 method ("inspecting seeds on the light") outperformed the M1 ("extracting the seeds one by one") by 3.33 times in our study in terms of time spent.

As for the comparison of the methods in terms of cost per fruit opening, M3 was found to be the cheapest ($3.81) and M2 being the most expensive ($40.62) method in our study. Likewise, Baktemur (2009) also determined the method of M3 ("inspecting seeds on the light") to be the most economic one with a different economic analysis parameter.

These values are crucial with respect to revealing the ability of fast, economic and practical usage of irradiated pollen technique for the commercial and scientific studies in which intensive breeding programs are maintained. While initially, the light source used for this purpose was a mammography device, today it turned into mostly a mechanism with light box developed by each laboratory's own facilities. In fact, a standard light source which can be used for this purpose has not been developed yet. So, the studies using nonstandardized light source in different laboratories will not give similar results even if all the conditions in the laboratories are the same. Therefore, development of the standardized commercial devices having appropriate light type and intensity is indeed very important for detecting haploid embryos in irradiated pollen technique studies.

In summary, the application of irradiated pollen technique in a more practical way is needed in breeding programs. In this study, the "inspecting seeds on the light" method was shown to be the most practical, economic and the fastest to detect and rescue haploid embryos in irradiated pollen technique. Additionally, the current report is the second one in the literature evaluating different embryo rescue methods in melon in a comparative way with regard to the time spent and the cost. This study also presented promising results that can be applied for other Cucurbitaceae species for the prospective haploid studies.

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