Meristem culture of two sweet cherry cultivars cvs. "Bing" and "Dovomras"

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Key words: Prunus avium, Initiation phase, Survival percent.

Abstract

The regeneration through meristem culture is an advanced biotechnological technique which is a very useful method for obtaining virus-free plants. In this study the effect of three media contains WPM, MS and QL and three different growth regulators combinations (0, 0.5 and 1 mgL⁻¹ BA, 0.1 mgL⁻¹ GA₃ and 0.1 mgL⁻¹ IBA) were investigated on regeneration of two sweet cherry cultivars cvs. "Bing" and "Dovomras" in spring season. All experiments were arranged in completely randomized designed. Each treatment contained three replicates. Explants were soaked in 100 mgL⁻¹ ascorbic acid and 150 mgL⁻¹ citric acid for one hour before surface sterilization to prevent browning during in vitro culture. The meristem explants (0.5-0.7 mm) cultured in media and maintained at 26°C under a 16 hr-light/8 hr-dark with a light intensity of 2000-3000 lux from white fluorescent light. After six weeks, survival, necrosis and contamination percent were studied. The results showed that WPM medium induced the most survival percent in both cultivars. MS medium decreased the survival percent and made the highest necrosis percent in both cultivars. Cultivars showed different responses to concentration of BA, so that, "Bing" cultivar in 1 mgL⁻¹ BA and "Dovomras" in 0.5 mgL⁻¹ BA had the best response (55.53-38.96%). The results also showed that "Bing" were more stable and had a higher survival percent than "Dovomras".

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Introduction
Sweet cherry (Prunus avium L.) is economically important member of the Rosaceae family, which is mainly grown for fresh consumption. In addition, the fruits are suitable for processing, e.g. for different candy and milk products, canning, to produce juice, liqueur and jam (Granger, 2004). Due to its suitable weather, Iran is the third biggest sweet cherry producer in the world, producing 200,000 tons per year (FAOSTAT, 2012). In order to overcome difficulties in vegetative propagation of sweet cherry cultivars and clones, methods of micropropagation were developed in past years (Hammatt, 1999). Micropropagation systems for Prunus avium were mostly developed and tested for genotypes aimed at forestry and timber industry (Ďurkovič, 2006) or for production of rootstocks and Scions (Erbenova et al., 2001). The interest for producing virus-free plants increased constantly based on the well known fact that most often is difficult to cure and restore the health of infected plants. The plants obtained from meristem cultures can be directly used, but most often they are used as mother plants for producing healthy planting material by the conventional vegetative propagation. However, we found that the efficiency of virus elimination is always higher when meristem explants with a size of 0.5-0.7 mm are used (Isac et al., 2010).

It was proved that different genotypes of sweet cherry do not respond in the same way during establishment in vitro (Erbenova et al., 2001). Success or failure in the micropropagation of fruit trees depends on the condition of plant material at the time of collection of the cuttings from grown trees in the field. This is mainly because the physiological conditions of plant tissues would depend on both the environmental conditions and the location of tissue obtained from plants (Bonga and Adercas 2002). The optimum explant collection time was the time when the shoots tend to decrease their growth percent (Mert and Soylu, 2010). However, the culture medium types and concentrations of growth regulators have been critical (Chakrabarty et al., 2003). Hu and Wang (1983) recommended to treat explants with ascorbic acid and citric acid to prevent browning phenomenon. Probably increasing the ascorbic acid concentration at the beginning of treatments can prevent the browning. Collection time of the explants and BAP level can also affect the browning percent (Mert and Soylu, 2010). Oztürk (2004) reported that optimum BAP concentrations were 0.5 mgL⁻¹ in this situation. Soliman (2012) reported that the most survival percent of meristems in Prunus armeniaca cultivar "El- Hamavey" were obtained in WPM medium supplement with 1 mgL⁻¹ zeatin and 0.1 mgL⁻¹ IAA in the presence of 100 mgL⁻¹ ascorbic acid and 150 mgL⁻¹ citric acid of explants taken in spring compared to the other season. Perez-Tornero & Burgos (2000) studied factors affecting in vitro propagation of several apricot cultivars with WPM medium were contained between 1.78 μM and 3.11 μM BA with different concentrations of IBA. Sugiiue et al. (1986) reported 1/2 MS or WPM medium was suitable for the culture of Japanese persimmon. Clapa (2007) also reported that survival percent in meristem culture of "Rhododendron" was 60 % when used WPM medium supplemented with 5 mgL⁻¹ 2ip although this percent was very low in MS medium. Salami et al. (2005) reported that cultivars showed differences response to BA concentrations in Vitis vinifera cvs so that, "Shahrudi" cultivar in 1 mgL⁻¹ BA and "Bidane" cultivar in 0.5 mgL⁻¹ BA had the best resonses. Mozafari and Bahramnejad (2010) said that the best medium culture to regeneration Sweet cherry cultivars cvs. "Camarosa" and "Selva" was MS medium supplemented with 1 mgL⁻¹ BA, 0.05 mgL⁻¹ GA3 and 0.05 mgL⁻¹ IBA. There is a little information about meristem culture of Sweet cherry cultivars, besides "Bing" and "Dovomras" cultivars are very important commercially.

The aim of the present study was to investigation of possibility producing of plantlets in Prunus avium cv. "Bing" and "Dovomras" by meristem culture.

Materials and methods
Plant material and explants preparation
Shoot tip explants were taken from mature Sweet cherry trees cvs. "Bing" and "Dovomras" and stored at
5°C. explants were washed with tap water for ten minutes and were soaked in 100 mgL⁻¹ ascorbic acid and 150 mgL⁻¹ citric acid for one hour before surface sterilization followed by 10 min to prevent browning during in vitro culture. Plant materials were washed with 70% ethanol indispensable for the application of biotechnological and 15 min immerged in 1.0% NaOCl. Finally shoot tips were rinsed three times with sterile water(Tioleneve, 1993).

Meristem excisions and planting on the culture medium

All work was done in a laminar air flow hood under sterile conditions. Meristem tips were dissected from disinfected shoot tips under stereomicroscope (SZ6045TR, Olympus Optical Co. Ltd., Tokyo, Japan). The meristem tip explants, composed of the apical dome and a few leaf primordia, were then excised and explanted. The explant size averaged from 0.5-0.7 mm.

Screening of Basal Medium for Meristem Tip Culture

Three media were used for meristem culture: MS (Murashig & Skoog, 1962), WPM (Lloyd & Mccown, 1980) and QL (Quoirin and Lepoivre, 1977) basal salt medium. All media were supplemented with 0.1 mgL⁻¹ IBA, 0.1 mgL⁻¹ GA3, three different concentration of BA (0, 0.5 and 1 mgL⁻¹), 1mgL⁻¹ Thiamine, 1 mgL⁻¹ Nicotinic acid, 0.1 mgL⁻¹ Biotin, 0.01 mgL⁻¹ Folic acid, 1 mgL⁻¹ P-aminobenzoic acid, 0.1 mgL⁻¹ Riboflavin, 0.5 mgL⁻¹ Ca-pantothenate (Perez-Tornero & Burgos, 2007), 3% sucrose and 6.7 gL⁻¹ Agar-Agar and the pH was adjusted to 5.7±0.1 (Table 1). Media was dispensed into 25 x 150 mm culture tubes, which were covered with permeable membrane caps and sterilized at 121°C for 20 min. fifteen explants were used for each medium. In all experiments, cultures were maintained at 26°C under a 16 hr-light/8 hr-dark with a light intensity of 2000-3000 lux from white fluorescent light. To avoid interference from phenolic compounds, meristems were kept in the dark for 1 week. After 45 days of culture, survival, necrosis and contamination percent were determined.

Statistical analysis

All experiments were arranged in completely randomized designed. Each treatment contained three replicates. Significant differences among the various treatments were compared using Duncan’s Multiple Rang Tests (Snedecor and Cochran, 1986).

Result

The results of screening for an optimal basal medium on meristem culture of Prunus avium cvs. "Bing" and "Dovomras" are shown in fig 1. The highest survival percent of meristem tips was 66.2% on the WPM medium in "Bing" cultivar and 49.96% in "Dovomras" cultivar (Fig 1). Our result showed that WPM medium (66.2-49.96%) was better than QL (33.43-22.43%) and MS (22.43-11.6%) media on the survival percent of meristems in "Bing" and "Dovomras" cultivars, respectively (Fig 1). BA treatments significantly increased the survival percent compared with the untreated media (control). Cultivars showed different responses to concentration of BA, so that, "Bing" cultivar in 1 mgL⁻¹ BA and "Dovomras" in 0.5 mgL⁻¹ BA had the best response (Table 1).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>BA (mgL⁻¹)</th>
<th>&quot;Bing&quot;</th>
<th>&quot;Dovomras&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mgL⁻¹ BA</td>
<td>27.98c</td>
<td>16.8d</td>
</tr>
<tr>
<td></td>
<td>0.5 mgL⁻¹ BA</td>
<td>44.4 b</td>
<td>38.96 b</td>
</tr>
<tr>
<td></td>
<td>1 mgL⁻¹ BA</td>
<td>61.10 a</td>
<td>28c</td>
</tr>
</tbody>
</table>

*Means with similar letter in each column are not significantly different at 5% level by Duncan’s multiple range test.

Mean comparision of the effects of media, plant growth regulators and cultivar were significant in 5%. The most survival percent observed in WPM medium supplemented with 1 mgL⁻¹ BA in "Bing" cultivar and the least one was in MS medium in control (0 mgL⁻¹ BA) in "Dovomras" cultivar(83.3-0%)(Table 2). The
results of basal medium, BA concentrations and cultivars on the necrosis percent of *Prunus avium* L. cvs. "Bing" and "Dovomras" were significant in 5% (Table 3). The most necrosis percent (83.3%) was observed in MS medium in "Dovomras" cultivar(Table 3). The lowest necrosis percent (0%) was observed in WPM medium complemented with 1 mgL⁻¹ BA in "Dovomras" cultivar. The result of basal medium, BA concentrations and cultivars on the contamination percent of *Prunus avium* L. cvs. "Bing" and "Dovomras" were significant in 5% (Table 4). contamination percent generally was low and the highest amount (33.3%) obtained in MS and QL media in control (Table 4).

**Table 2.** The effects of media and BA concentrations on the survival percent in *Prunus avium* cvs. "Bing" and "Dovomras".

<table>
<thead>
<tr>
<th>Media</th>
<th>WPM</th>
<th>MS</th>
<th>QL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&quot;Bing&quot;</td>
<td>&quot;Dovomras&quot;</td>
<td>&quot;Bing&quot;</td>
</tr>
<tr>
<td>0 mgL⁻¹ BA</td>
<td>50 c</td>
<td>33.3 d</td>
<td>33.3 d</td>
</tr>
<tr>
<td>0.5 mgL⁻¹ BA</td>
<td>66.6 b</td>
<td>61.6 b</td>
<td>33.3 d</td>
</tr>
<tr>
<td>1 mgL⁻¹ BA</td>
<td>83.3 a</td>
<td>50 c</td>
<td>50 c</td>
</tr>
</tbody>
</table>

*Means with similar letter in each column are not significantly different at 5% level by Duncan’s multiple range test.

**Table 3.** The effects of media and BA concentrations on the necrosis percent in *Prunus avium* cvs. "Bing" and "Dovomras".

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<th>Media</th>
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<th>MS</th>
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</tr>
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<tbody>
<tr>
<td></td>
<td>&quot;Bing&quot;</td>
<td>&quot;Dovomras&quot;</td>
<td>&quot;Bing&quot;</td>
</tr>
<tr>
<td>0 mgL⁻¹ BA</td>
<td>50 c</td>
<td>50 c</td>
<td>66.6 b</td>
</tr>
<tr>
<td>0.5 mgL⁻¹ BA</td>
<td>17 e</td>
<td>17 e</td>
<td>66.6 b</td>
</tr>
<tr>
<td>1 mgL⁻¹ BA</td>
<td>0 f</td>
<td>50 c</td>
<td>50 c</td>
</tr>
</tbody>
</table>

*Means with similar letter in each column are not significantly different at 5% level by Duncan’s multiple range test.

**Discussion**

Explants were collected in summer on the basis of Das & Mitra (1990) who found that explants collected from new shoots in the summer exhibited the highest survival percentage compared to explants collected from late period of the growing season. The results of screening for an optimal basal medium on meristem culture of *Prunus avium* cvs. "Bing" and "Dovomras" are shown in fig 1. Apex size was 0.5-0.7 mm on the basis of the Isac et al (2010) who found that efficiency of virus elimination is always higher when meristem explants with a size of 0.5-0.7 mm are used. The highest survival percent of meristem tips was 66.2% on the WPM medium in "Bing" cultivar and 49.96% in "Dovomras" cultivar (Fig 1). Our result showed that WPM medium (66.2-49.96%) was better than QL (33.43-22.43%) and MS (22.43-11.6%) media on the survival percent of meristems in "Bing" and "Dovomras" cultivars, respectively (Fig 1). This result is agreemented with Sugiuire et al. (1986) that reported 1/2 MS or WPM medium was suitable for the culture of Japanese persimmon meristems and with Clapa (2007) that reported survival percent in meristem culture of "Rhododendron" was 60 % when used WPM medium although this percent was very low in MS medium. Nitrogen concentration of WPM medium is less than that of MS medium, therefore the nitrogen level may have been excessive in MS media.
BA treatments significantly increased the survival percent compared with the untreated media (control). Cultivars showed different responses to concentration of BA, so that, "Bing" cultivar in 1 mgL$^{-1}$ BA and "Dovomras" in 0.5 mgL$^{-1}$ BA had the best response (Table 1). These result is agreement with Salami et al. (2005) that reported cultivars showed differences response to BA concentrations in Vitis vinifera cvs so that, "Shahrudi" cultivar in 1 mgL$^{-1}$ BA and "Bidane" cultivar in 0.5 mgL$^{-1}$ BA had the best response. The poor response of survival ability was noticed in other concentrations and different combinations of the growth regulators (Table 1). Mean comparison of the effects of media, plant growth regulators and cultivar were significant in 5%. The most survival percent observed in WPM medium supplemented with 1 mgL$^{-1}$ BA in "Bing" cultivar and the least one was in MS medium in control (0 mgL$^{-1}$ BA) in "Dovomras" cultivar (83.3-0%) (Table 2).

**Table 4.** The effects of media and BA concentrations on the contamination percent in Prunus avium cvs. "Bing" and "Dovomras".

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>&quot;Bing&quot;</td>
<td>0 d</td>
<td>0 d</td>
<td>33.3 a</td>
</tr>
<tr>
<td>&quot;Dovomras&quot;</td>
<td>17 b</td>
<td>0 d</td>
<td>0 d</td>
</tr>
<tr>
<td>&quot;Bing&quot;</td>
<td>17 b</td>
<td>0 d</td>
<td>0 d</td>
</tr>
<tr>
<td>&quot;Dovomras&quot;</td>
<td>0 d</td>
<td>0 d</td>
<td>33.3 a</td>
</tr>
</tbody>
</table>

*Means with similar letter in each column are not significantly different at 5% level by Duncan's multiple range test.

![Fig 1. Effect of media on the survival percent of Prunus avium cvs. "Bing" and "Dovomras".](image)

The results of basal medium, BA concentrations and cultivars on the contamination percent of Prunus avium L. cvs. "Bing" and "Dovomras" were significant in 5% (Table 4). The contamination percent generally was low and the highest amount (33.3%) obtained in MS and QL media in control (Table 4).

**Acknowledgment**

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**References**


Clapa DF. 2007. Tissue culture and ex vitro
acclimation of Rhododendron sp., Buletinul USAMV-CN.


FAOSTAT. 2012. FAOSTAT database result. [http://faostat.fao.org/]


Hammatt N. 1999. Delayed flowering and reduced branching in micropropagated mature wild cherry (Prunus avium L.) compared with rooted cuttings and seedlings. Plant Cell Reports 18, 478–484. [http://dx.doi.org/10.1007/s00122-003-1426-6].


