Meristem culture of *Prunus avium* cvs. "Siyah-e-Mashhad" and "Takdane"

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**ABSTRACT**

The present trial aimed to evaluate the effect of three media including WPM, MS and modified QL and three different hormone combinations (0, 0.5 and 1 mgL⁻¹ BAP) with 0.1 mgL⁻¹ GA3 and 0.1 mgL⁻¹ IBA on regeneration of two sweet cherry cultivars cvs "Siyah-e-Mashhad" and "Takdane". All experiments were arranged in completely randomized designed. Each treatment contained three replicates. The meristem explants were introduced in culture media supplemented with different hormonal combinations and maintained in a growth room at a 16h photoperiod (36 μmol.m⁻².s⁻¹), 25 ± 1°C. After six weeks, Survival, necrosis and contamination percent with the number of leaf were studied. The results showed that highest survival rate was in modified QL medium complemented with 1 mgL⁻¹ BAP and the least one was in MS medium without BAP treatments for both cultivars. Necrosis rate was high in MS (100-83.3%) in control for "Takdane" and "Siyah-e-Mashhad" cultivars, respectively that don’t have any differences with 0.5 mgL⁻¹ BAP. The most Leaf number (12) observed in modified QL medium supplemented with 1 mgL⁻¹ BAP in "Takdane" cultivar and the least one (2) was in MS medium without BAP application (control) in "Siyah-e-Mashhad" cultivar.

**INTRODUCTION**

Sweet cherry (*Prunus avium* L.) is a deciduous tree originated around the Black and Caspian Seas. It is a diploid species (2n = 16), allogamous, generally self-incompatible, that is mainly cultured for its edible fruit. Sweet cherries are used for fresh consumption and to produce jam, jelly, stewed fruit, marmalade, syrup and several types of soft drinks (Perez-Sanchezi et al. 2010). Due to its suitable weather, Iran is the third biggest sweet cherry producer in the world, producing 140, 000 tons per year (FAO 2017). The use of tissue culture in the regeneration and commercial propagation of economically important plants is a comparative recent and radical development. Advances in biotechnology provided new methods for rapid production of high quality, disease-free and uniform planting material. Biotechnological tools like in vitro culture and micropropagation offer a valuable alternative in fruit trees propagation studies, virus control and management of genetic resources. 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 (Isac et al. 2010). 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 rate (Mert and Soylu 2010). However, the culture medium types and concentrations of growth regulators have been critical (Chakrabarty et al. 2003). Also, Soliman (2012) reported that the most survival rate of

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meristems in *Prunus armeniaca* cultivar "El-Hamavay" 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. Also, Ozturk (2004) reported that using optimum BAP concentrations (0.5 mgL⁻¹) decreased browning rate. Mozafari and Bahramnejad (2010) said that the best medium culture to regeneration strawberry 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. Perez-Tornero and Burgos (2007) studied factors affecting in vitro propagation of several apricot cultivars with modified QL (2007) basal salt medium. All media were supplemented with 0.1 mgL⁻¹ IBA, 0.1 mgL⁻¹ GA3, three different concentration of BAP (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-amino benzoic acid, 0.1 mgL⁻¹ Riboflavin, 0.5 mgL⁻¹ Ca-pantothenate (Perez-Tornero and 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 ratios and also leaf number were determined.

### Statistical Analysis

All experiments were arranged in completely randomized designed. Each treatment contained

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**Table 1. Experimental variants used in initiation phase**

| Media                        | Woody Plant Medium | Murashig & Skoog | Modified Quoirin & Lepoivre |
|------------------------------|--------------------|------------------|-----------------------------|
| Indolbuteric acid            | 0.1 mgL⁻¹          | 0.1 mgL⁻¹        | 0.1 mgL⁻¹                   |
| Gibberellic acid             | 0.1 mgL⁻¹          | 0.1 mgL⁻¹        | 0.1 mgL⁻¹                   |
| Benzyl amino purine          | 0.0, 0.5 and 1 mgL⁻¹ | 0.0, 0.5 and 1 mgL⁻¹ | 0.0, 0.5 and 1 mgL⁻¹        |
| Thiamine                     | 1 mgL⁻¹            | 1 mgL⁻¹          | 1 mgL⁻¹                     |
| Nicotinic acid               | 1 mgL⁻¹            | 1 mgL⁻¹          | 1 mgL⁻¹                     |
| Biotin                       | 0.1 mgL⁻¹          | 0.1 mgL⁻¹        | 0.1 mgL⁻¹                   |
| Folic acid                   | 0.01 mgL⁻¹         | 0.01 mgL⁻¹       | 0.01 mgL⁻¹                  |
| P-amino benzoic acid         | 1 mgL⁻¹            | 1 mgL⁻¹          | 1 mgL⁻¹                     |
| Riboflavin                   | 0.1 mgL⁻¹          | 0.1 mgL⁻¹        | 0.1 mgL⁻¹                   |
| Ca-pantothenate              | 0.5 mgL⁻¹          | 0.5 mgL⁻¹        | 0.5 mgL⁻¹                   |
| Sugar                        | 30 gL⁻¹            | 30 gL⁻¹          | 30 gL⁻¹                     |
| Agar-Agar                    | 6.7 gL⁻¹           | 6.7 gL⁻¹         | 6.7 gL⁻¹                    |
| PH                           | 5.7±0.1            | 5.7±0.1          | 5.7±0.1                     |

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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 (SZ604STR, 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 (Isac et al. 2010).

### Screening of Basal Medium for Meristem Tip Culture

Three media were used for meristem culture: MS (Murashig and Skoog 1962), WPM (Lloyd and Mccown 198) and modified 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 BAP (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-amino benzoic acid, 0.1 mgL⁻¹ Riboflavin, 0.5 mgL⁻¹ Ca-pantothenate (Perez-Tornero and 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 ratios and also leaf number were determined.

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three replicates. Significant differences among the various treatments were compared using Duncan's Multiple Rang Tests (Snedecor and Cochran, 1986).

RESULTS AND DISCUSSION

A biotechnological alternative to obtain large quantities of healthy plants is the isolation of meristematic tissue, since this is generally free of viruses because its active cell division reduces differentiation of vascular tissues (García-Gonzáles et al., 2010). Thus, meristematic tissue culture is an appealing technique to eliminate pathogenic bacteria, fungus, and viruses carried by adult plants. However, a number of constraints need to be overcome in order to facilitate meristem isolation and establishment in vitro conditions including: reducing the release of phenolic compounds from the tissues into the culture medium, and appropriate environmental conditions, such as suitable temperatures (Chien-Ying et al. 2009).

The results of screening for an optimal basal medium on meristem culture of Prunus avium cvs. "Siyah-e-Mashhad" and "Takdane" are shown in fig 1. The highest survival rate of meristem tips was 49.9% on the QL medium in "Siyah-e-Mashhad" cultivar and 33.4% in "Takdane" cultivar (Fig 1). Our result showed that QL medium (49.9-33.4%) was better than WPM (35.2-22.4%) and MS (22.4-5.7%) media on the survival rate of meristems in "Siyah-e-Mashhad" and "Takdane" cultivars, respectively (Figure 1). Perez-Tornero and Burgos (2007) reported that modified QL medium ensure a high meristem survival rate in the apricot cultivars and is agree with our result.

The results of basal medium, BAP concentrations and cultivars on the necrosis rate of Prunus avium L. cvs. "Siyah-e-Mashhad" and "Takdane" were significant in 5% (Table 3). The most necrosis rate (100%) was observed in MS medium in 0 mgL\(^{-1}\) BAP. The lowest necrosis rate (17%) was observed in QL medium supplemented with 1 mgL\(^{-1}\) BAP in "Siyah-e-Mashhad" cultivar. Clapa (2007) reported that browning rate in meristem culture of "Rhododendron" was very low in MS medium and is agree with our result.

The results of basal medium, BAP concentrations and cultivars on the contamination rate of Prunus avium L. cvs. "Siyah-e-Mashhad" and "Takdane" were significant in 5% (Table 4). The most contamination rate was obtained in QL media supplemented with different concentrations of BAP in "Takdane" cultivar (Table 4).

Phenolic oxidation is a problem prevalent in the growth and propagation of established in vitro plants and has both an environmental and genetic component. Oxidation of “in vitro established” explants can be controlled by modifying the environmental conditions of cultivation and the management of explants (Husain and Anis, 2009), or through the addition of antioxidants to the nutrient medium (Bhatia and Ashwath, 2008). Quiroz et al (2017) reported that BAP in the media reduces oxidation in Chilean strawberry and is agree with our result.

Mean comparison of the effects of media on the leaf number in "Siyah-e-Mashhad" and "Takdane" cultivar are shown in Figure 2. The leaf number varied between 3.3 in Ms medium in "Siyah-e-Mashhad" cultivar to 9.0 in QL medium in "Takdane" cultivar, respectively (Figure 2). The result of the effects of BAP concentrations on the leaf number are shown in Table 5. The leaf number varied between 3 in "Siyah-e-Mashhad" cultivar without using BAP to 9.66 in "Takdane" cultivar with application of 1 mgL\(^{-1}\) BAP, respectively (Table 5). The highest leaf number (12) was in "Takdane" cultivar in QL medium complemented with 1 mgL\(^{-1}\) BAP and the least one (2) was in "Siyah-e-Mshhad" cultivar in MS medium without using BAP (Table 6 and Figure 3).
Table 2. The effects of media and BAP concentrations on the survival rate in *Prunus avium* cvs. "Siyah-e-Mashhad" and "Takdane"

| Media | WPM | MS | QL |
|-------|-----|----|----|
| Cultivars | "Siyah-e-Mashhad" | "Takdane" | "Siyah-e-Mashhad" | "Takdane" | "Siyah-e-Mashhad" | "Takdane" |
| 0 mgL\(^{-1}\) BAP | 22.43d\* | 17.00 e | 17.00 e | 0.10 f | 33.30 c | 17.00 e |
| 0.5 mgL\(^{-1}\) BAP | 33.30c | 17.00 e | 17.00 e | 0.10 f | 50.00 b | 33.30 c |
| 1 mgL\(^{-1}\) BAP | 50.00 b | 33.30 c | 33.30 c | 17.00 e | 66.60 a | 50.00 b |

*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 BAP concentrations on the Necrosis rate in *Prunus avium* cvs. "Siyah-e-Mashhad" and "Takdane"

| Media | WPM | MS | QL |
|-------|-----|----|----|
| Cultivars | "Siyah-e-Mashhad" | "Takdane" | "Siyah-e-Mashhad" | "Takdane" | "Siyah-e-Mashhad" | "Takdane" |
| 0 mgL\(^{-1}\) BAP | 55.50d\* | 83.30 b | 83.30 b | 100 a | 66.60 c | 50.00 d |
| 0.5 mgL\(^{-1}\) BAP | 66.60c | 83.30 b | 83.30 b | 100 a | 50.00 d | 33.30 e |
| 1 mgL\(^{-1}\) BAP | 33.30 e | 66.60 c | 66.60 c | 83.30 b | 17.00 f | 17.00 f |

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

Table 4. The effects of media and BAP concentrations on the Contamination rate in *Prunus avium* cvs. "Siyah-e-Mashhad" and "Takdane"

| Media | WPM | MS | QL |
|-------|-----|----|----|
| Cultivars | "Siyah-e-Mashhad" | "Takdane" | "Siyah-e-Mashhad" | "Takdane" | "Siyah-e-Mashhad" | "Takdane" |
| 0 mgL\(^{-1}\) BAP | 22.43b\* | 0.10 d | 0.10 d | 0.10 d | 0.10 d | 33.30 a |
| 0.5 mgL\(^{-1}\) BAP | 0.10 d | 0.10 d | 0.10 d | 0.10 d | 0.10 d | 33.30 a |
| 1 mgL\(^{-1}\) BAP | 17.00 c | 0.10 d | 0.10 d | 0.10 d | 17.00 c | 33.30 a |

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

Table 5. The effects of BAP concentrations on the Leaf number in *Prunus avium* cvs. "Siyah-e-Mashhad" and "Takdane"

| Media | WPM | MS | QL |
|-------|-----|----|----|
| Cultivar | "Siyah-e-Mashhad" | "Takdane" | "Siyah-e-Mashhad" | "Takdane" | "Siyah-e-Mashhad" | "Takdane" |
| 0 mgL\(^{-1}\) BAP | 3.00 i | 4.00 h | 2.00 j | 3.00 i | 4.00 h | 6.00 f |
| 0.5 mgL\(^{-1}\) BAP | 4.00 h | 9.00 c | 3.00 i | 6.00 f | 6.00 f | 9.00 c |
| 1 mgL\(^{-1}\) BAP | 6.00 f | 10.00 b | 5.00 g | 7.00 e | 8.00 d | 12.00 a |

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

Table 6. The effects of media and BAP concentrations on the leaf number in *Prunus avium* cvs. "Siyah-e-Mashhad" and "Takdane"

| Media | WPM | MS | QL |
|-------|-----|----|----|
| Cultivars | "Siyah-e-Mashhad" | "Takdane" | "Siyah-e-Mashhad" | "Takdane" | "Siyah-e-Mashhad" | "Takdane" |
| 0 mgL\(^{-1}\) BAP | 3.00 i | 4.00 h | 2.00 j | 3.00 i | 4.00 h | 6.00 f |
| 0.5 mgL\(^{-1}\) BAP | 4.00 h | 9.00 c | 3.00 i | 6.00 f | 6.00 f | 9.00 c |
| 1 mgL\(^{-1}\) BAP | 6.00 f | 10.00 b | 5.00 g | 7.00 e | 8.00 d | 12.00 a |

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

Figure 3. The effects of media (WPM, QL and MS) and BAP concentrations (0, 0.5 and 1 mgL\(^{-1}\) from left to right, respectively) on the survival rate and leaf number in *Prunus avium* cvs. "Siyah-e-Mashhad" and "Takdane"
Perez-Tornero and Burgos (2007) reported that modified QL medium ensure the high development of a rosette of leaves in the apricot cultivars and Erbenova et al. (2001) reported that different genotypes of sweet cherry do not respond in the same way during establishment in vitro and is agree with our results.

CONCLUSIONS

Meristem culture showed that can be an efficient tool for eliminating virus from infected plants, giving the possibility to produce disease free propagation material. In both cultivars, the addition of BAP (1 mgL⁻¹) into the modified QL medium improved the efficiency of survival rate and plant regeneration from isolated meristems.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

REFERENCES

Bonga J.M. Van Adercas P. (2002) Tissue culture of woody plants. Translated by Arvin MJ Shahid Bahonar University Publications. Kerman, Iran.

Bhatia P. Ashwath N. (2008) Improving the quality of in vitro culture shoots of tomato. Biotechnology. 7: 188–193. doi: 10.3923/biotech.2008.188.193.

Chakrabarty D. Hahn E.J. Yoon Y.J. Paek. K.Y. (2003) Micropropagation of apple rootstock M.9 EMLA using bioreactor. Journal of Horticultural Science and Biotechnology, 65(1): 605-609.

Chien-Ying K. Al-Abdulkarim A.M. Al-Jowid S.M. Al-Baiz A. (2009) An effective disinfection protocol for plant regeneration from shoot tip cultures of strawberry. African Journal of Biotechnology, 8: 2611–2615.

Clapa D.F. (2007). Tissue culture and ex vitro acclimation of Rhododendron sp., Buleinul USAMY-CN.

Erbenova M. Papr štein F. Sedl Ak J. (2001) In vitro propagation of dwarfed rootstocks for sweet cherry. Acta Horticulturae, 560: 477–480.

FAO. (2017) On-line crop database ECOCROP. URL: http://ecocrop.fao.org/ecocrop/srv/en/cropSearchForm.

García-Gonzáles R. Quiroz K. Caligari P.D.S. Carrasco B. (2010). Plant tissue culture: current status, opportunities and challenges. Ciencia e Investigación Agraria. 37: 5–30. doi: 10.4067/S0718-16202010000300001.

Husain M.K. Anis M. (2009) Rapid in vitro multiplication of Melia azedarach L. (a multipurpose woody tree) Acta Physiologica Plantarum, 31:765–772. doi: 10.1007/s11738-009-0290-7.

Isac V. Coman T. Marinescu L. Isac M. Teodorescu A. Popescu A. Coman M. Plopa C. (2010) Achievements and Trends in the Use of Tissue Culture for the Mass Propagation of Fruit Plants and Germplasm Preservation at the Research Institute for Fruit Growing, Pitesti, Roman. Romanian Biotechnological Letters.

Jakab I. Zs. Panfil D. Clapa D. Fira A. (2008) In vitro regeneration and meristem culture of Prunus domestica cv. Bulletin UASVM, Horticulture, 65 (1): 126–131.

Lloyd G. McCown B. (1981) Commercially feasible micropropagation of mountain laurel, Kalmia latifoliaby use of shoot-tip culture. Combined Proceeding, International Plant Propagators Society, 30: 421–427.

Mert C. Soylu S. (2010) Shoot location and collection time effects on meristem tip culture of some apple rootstocks. Pakistan Journal of Botany, 42 (1): 549-557.

Mozaafari A. Bahramnejad. B. (2010) The effects of medium and hormones mixture on the morphological changes of Fragaria annanassa by meristem culture. Life Technology, 9(2): 17-29.

Murashige T. Skoog F. (1962) A revised medium for rapid growth and bioassay with tobacco culture. Journal of Plant Physiology, 15: 473-497. http://dx.doi.org/10.1111/j1399-3054

Öztürk Ö. (2004) Micropropagation of some apple clonal rootstocks. MS Thesis. Uludag University, Institute Science, Bursa. 42.

Perez-Sanchez R. Gomez-Sanchez M.A. Morales-Corts M.R. (2010) Description and quality evaluation of sweet cherries cultured in Spain. Journal of Food Quality, 33: 490-506. DOI: 10.1111/j.1745-4557.2010.00339.x

Pérez-Tornero O. Burgos L. (2007) Apricot micropropagation. In: Jain, S.M. and Haggman (eds.), Protocols for micropropagation of woody trees and fruits, 267-278.

Quoirin M. and Lepoivre P. (1977) Etude de milieux. Contaminants of adaptes aux cultures in vitro de Prunus. Acta Horticulturae, 78: 437-442.

Quiroz K.A. Berrios M. Carrasco B. Retamales J.B. Caligari P.D.S. García-Gonzáles R. (2017) Meristem culture and subsequent micropropagation of Chilean strawberry (Fragaria chiloensis (L.) Duch.). Journal of
Salami S. Ebadi H. Zamani Z. (2005) The effects of explant times, apex size and culture medium on the initiation phase of meristem culture in Vitis vinifera cvs. Bidane and Shahrudi. Investigation in Agricultural and Horticultural Science, 67: 72-81.

Snedecor G.M. Cochran W.G. (1986) Statistical Methods. 9 Ed., The Iowa State University, Press. Th. Amer. Iowa, U.S.A. 07 P.

Soliman H. (2012). In vitro propagation of Apricot (Prunus armeniaca L.) and assessment of genetic stability of micropropagated plants using RAPD analysis. World Applied Sciences Journal, 19(5): 674-687. http://dx.doi.org/105829/idosi.wasj.2012.19.5.2770.

Tioleneve V.M. (1993) Regeneration of Front Crops from Somatic Tissues, 1st edition, Pushino 173.