



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



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ABSTRACT

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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-Sanchez 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 Soyly 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-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. 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 medium were contained between 2.2 µM and 8.8 µM BA with different concentrations of IBA. Jakab et al. (2008) reported that the optimum BAP hormone concentration in meristem culture of *Prunus domestica* L. was found to be 0.7 mgL⁻¹ both for initiation and multiplication.

The aim of the present study was to investigation of possibility producing of plantlets in *Prunus avium* cv. "Siyah-e-Mashhad" and "Taktane" by meristem culture.

MATERIALS AND METHODS

Plant material and explants preparation

Shoot tip explants were taken from mature Sweet cherry trees cvs. "Siyah-e-Mashhad" and "Taktane" is obtained between January and March, when buds starting to swell from shoots in dormancy 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 immersed 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 (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-aminobenzoic 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

Table1. 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
Gibberelic acid	0.1 mgL ⁻¹	0.1 mgL ⁻¹	0.1 mgL ⁻¹
Benzyl amino purine	0, 0.5 and 1 mgL ⁻¹	0, 0.5 and 1 mgL ⁻¹	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-panthotenate	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

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-González 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 *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 "Taktane" 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 "Taktane" 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 "Taktane" 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.

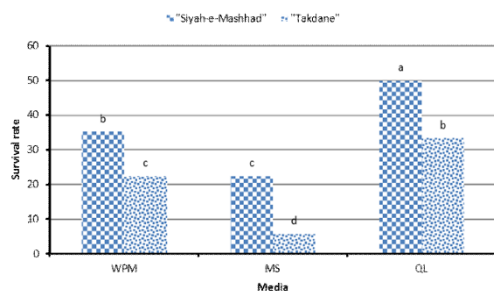


Figure 1. Effect of media on the survival rate of *Prunus avium* cvs. "Siyah-e-Mashhad" and "Taktane"

Mean comparison of the effects of media, plant growth regulators and cultivar on the survival rate were significant in 5%. The most and the least survival rate observed in QL medium supplemented with 1 mgL⁻¹ BAP in "Siyah-e-Mashhad" cultivar and MS medium in concentration of 0 and 0.5 mgL⁻¹ BAP in "Taktane" cultivar (66.6-0.1%)(Table 2). Salami et al. (2005) reported that the most survival induced in "Shahrudi" cultivar in 1 mgL⁻¹ BA 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 "Taktane" 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 "Taktane" were significant in 5% (Table 4). The most contamination rate was obtained in QL media supplemented with different concentrations of BAP in "Taktane" 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 "Taktane" 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 "Taktane" 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 "Taktane" cultivar with application of 1 mgL⁻¹ BAP, respectively (Table 5). The highest leaf number (12) was in "Taktane" 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).

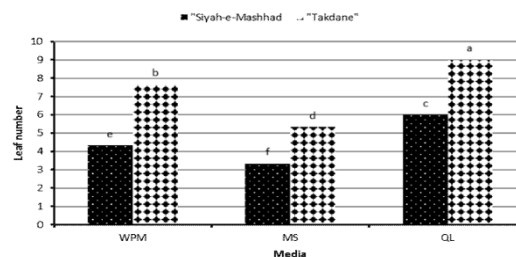


Figure 2. Effect of media on the leaf number of *Prunus avium* cvs. "Siyah-e-Mashhad" and "Taktane"

Table 2. The effects of media and BAP concentrations on the survival rate in *Prunus avium* cvs. "Siyah-e-Mashhad" and "Taktane"

Media	Survival rate (%)					
	WPM		MS		QL	
Cultivars	"Siyah-e-Mashhad"	"Taktane"	"Siyah-e-Mashhad"	"Taktane"	"Siyah-e-Mashhad"	"Taktane"
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 "Taktane"

Media	Necrosis rate (%)					
	WPM		MS		QL	
Cultivars	"Siyah-e-Mashhad"	"Taktane"	"Siyah-e-Mashhad"	"Taktane"	"Siyah-e-Mashhad"	"Taktane"
0 mgL ⁻¹ BAP	55.50d*	83.30 b	83.30 b	100 a	66.60c	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 "Taktane"

Media	Contamination rate (%)					
	WPM		MS		QL	
Cultivars	"Siyah-e-Mashhad"	"Taktane"	"Siyah-e-Mashhad"	"Taktane"	"Siyah-e-Mashhad"	"Taktane"
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 "Taktane"

Cultivar	"Siyah-e-Mashhad"		"Taktane"	
	BAP (mgL ⁻¹)		BAP (mgL ⁻¹)	
0 mgL ⁻¹ BAP	3.00 e		4.33 d	
0.5 mgL ⁻¹ BAP	4.33 d		8.00 b	
1 mgL ⁻¹ BAP	6.33 c		9.66 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 "Taktane"

Media	Leaf number					
	WPM		MS		QL	
Cultivars	"Siyah-e-Mashhad"	"Taktane"	"Siyah-e-Mashhad"	"Taktane"	"Siyah-e-Mashhad"	"Taktane"
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.

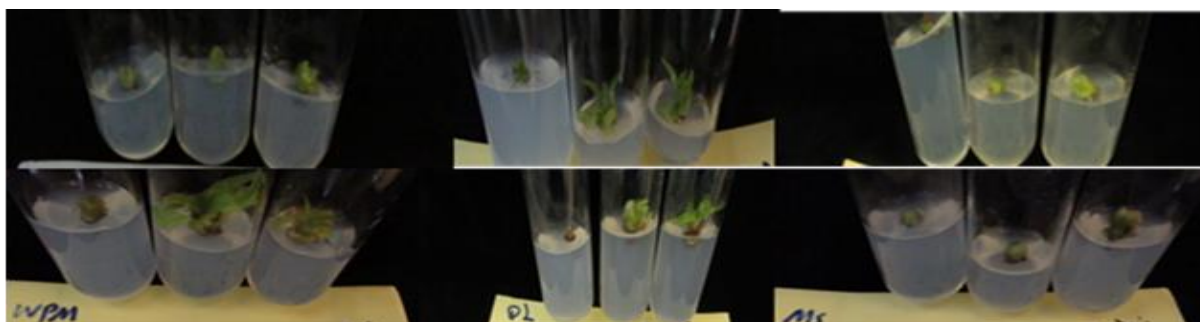


Figure 3. The effects of media (WPM, QL and MS) and BAP concentrations (0, 0.5 and 1 mgL⁻¹ from left to right, respectively) on the survival rate and leaf number in *Prunus avium* cvs. "Siyah-e-Mashhad" and "Taktane"

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.

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