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Initiation phase of meristem culture of *Prunus avium* cvs. "Hajyusefi" and "Zard"

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Abstract

This trial was carried out to determine the best medium and plant growth regulator concentrations on the initiation of meristem tips from sweet cherry trees cvs. "Hajyusefi" and "Zard". Meristems were isolated from actively growing plants in the spring and cultured on three kinds of basal media (MS, WPM and QL) supplement with three different concentrations of BA (0, 0.5 and 1 mgL^{-1}), 0.1 mgL^{-1} GA3 and 0.1 mgL^{-1} IBA. Explants were soaked in antioxidant solutions (100 mgL^{-1} ascorbic acid and 150 mgL^{-1} citric acid for 1 hours). The meristem explants (0.5-0.7 mm) cultured in medium and maintained in a growth room at a 16h - light/8 hr-dark with a light intensity of 2000-3000 lux from white fluorescent light. After six weeks, survival and necrosis ratios and leaf number were studied. Result showed that the highest survival rate and the low necrosis rate (83.3%) was in WPM medium supplemented with 0.5 mgL^{-1} BA for "Hajyusefi" cultivar. Necrosis occurred more often in vitro condition. Necrosis rate was high in MS (100-83.3%) for "Zard" and "Hajyusefi" cultivars, respectively. The most Leaf number (11) observed in WPM medium supplemented with 0.5 mgL^{-1} BA in "Hajyusefi" cultivar and the least one (2) was in MS medium without BA application (control) in "Zard" cultivar.

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Introduction

Prunus avium L. (cherry) is native of Iran (around of the Caspiansea), and one of the main stone fruits being cultivated for long time (Hartmann *et al.*, 1990). Due to its suitable weather, Iran is the third biggest sweet cherry producer in the world, producing 200, 000 tons per year (FAOSTAT, 2012). Traditional propagation of cherry is by seed and budding. There are some problems with the plant cultivation, such as laborious, poor and uniform germination, prolonged seedling emerging and disease susceptibility to mycoplasma (Hartmann *et al.*, 1990). The tissue culture propagation method provides a very good phytosanitary status and the final planting material will have high phytosanitary status (Hanzer and Weis, 1995). Beside the difficulty of the *in vitro* meristem or apex culture it must be stated that in the case of some cultivars considerably good results can be expected (Clapa *et al.,* 2007).The regeneration through meristem culture is an advanced biotechnological tehnique which is a very useful and valuable method and represents a key in the fruit stock material production chain. In the modern fruit planting material production system and in the pathogen elimination systems meristem culture occupies a central place (Jakab *et al.*, 2008). Meristem tips can easily be obtained from the actively growing shoot tips (Ozturk, 2004). Therefore, the spring or early summer days might be suitable for this purpose. Active growing season of the trees depends on the climatic conditions. For this reason, the optimum cutting time of the explants needs to be determined for different ecological conditions. In addition, the time to get the explants during day might also be important because CO2 fixation product varies according to the time of a day in the leaves during photosynthesis and sugar formation is enhanced from the morning through afternoon (Leopold and Kriedemann, 1975). The location of the explants e.g., terminal versus lateral shoots, can also affect the growth of the meristems and multiplication capability of the shootlets. It had been stated that isolate position on shoot formation from meristems can be an effective factor (Golasin & Radojevic, 1987). However, the culture medium types and concentrations of growth regulators have been critical and different concentrations of cytokinins (BA or BAP), gibberellins (GA3), or auxins (IAA, NAA, or IBA), have been used for various kinds of plant materials and various steps of the tissue cultures (Radmann *et al.*, 2002; 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 ratio (Mert and Soylu, 2010). Ozturk (2004) reported that optimum BAP concentrations were 0.5 mgL^{-1} in this respect. Soliman (2012) reported that the most survival rate 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. Also, Sugiure *et al.* (1986) reported 1/2 MS or WPM medium was suitable for the culture of Japanese persimmon. Jakab *et al.* (2008) reported that MS medium supplemented with 0.7 mgL^{-1} BAP and 0.1 mgL^{-1} IBA increased survival rate in *Prunus domestica* cultivar "Jubileu". Comlekcioglu *et al.* (2007) said that the most favourit result obtained with application of MS medium complemented with 0.5 mgL^{-1} BA, 0.2 mgL^{-1} GA3 and 0.1 mgL^{-1} IBA for fig cultivar "Bursa" in Turkey. 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. 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. Pruski *et al* (2000) and Ďurkovič (2006) worked with different *Prunus* species and reported that small curly shoots or poor leaf expansion on media induced with using higher concentration of BA.

The aim of the present study was to investigation of

possibility producing *of plantlets in Prunus avium* cv. "Hajhusefi" and "Zard" by meristem culture.

Materials and methods

Plant material and explants preparation

Shoot tip explants were taken from mature sweet cherry trees cvs. "Hajyusef" and "Zard" 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 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 cultured.

Screening of Basal Medium for Meristem Tip Culture

Three media were used for meristem culture: MS (Murashig and Skoog, 1962), WPM (Lioyd and Mccown, 1981) 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 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 and necrosis ratios and also leaf area 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. "Hajyusefi" and "Zard" are shown in fig 1. The highest survival rate of meristem tips was 55.5% on the WPM medium in "Hajyusefi" cultivar and 38.9% in "Zard" cultivar (Fig 1). Our result showed that WPM medium (55.5-38.96) was better than QL (38.9-2.43%) and MS (33.4-5.7%) media on the survival rate of meristems in "Hajyusefi" and "Zard" cultivars, respectively(Fig 1). BA treatments significantly increased the survival rate compared with the untreated media (control). The using of the 0.5 mgL^{-1} BA inhanced the survival rate in both cultivars (Fig 2). The poor response of survival ability was noticed in other concentrations and different combinations of the growth regulators (Fig 2). Mean comparision 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 WPM medium supplemented with 0.5 mgL^{-1} BA in "Hajusefi" cultivar and MS medium in concentraton of 0 and 1 mgL^{-1} BA in "Zard" cultivar(83.3-0%) (Table 2).

The results of basal medium, BA concentrations and cultivars on the necrosis rate of *Prunus avium* L. cvs. "Hajyusefi" and "Zard" were significant in 5% (Table 2). The most necrosis rate (100%) was observed in MS medium in 0 mgL^{-1} BA. Although application of 1 mgL^{-1} BA didn't showed differences with control and couldn't counteract with necrosis (Table 2). The lowest necrosis rate (17%) was observed in WPM

medium complemented with 0.5 mgL^{-1} BA in "Hajyusefi" cultivar.

Mean comparision of the effects of media on the leaf number in "Hajyusefi" and "Zard" cultivar are shown in Table 3. The leaf number changed between 5 in Ms medium in "Zard" cultivar to 9 in WPM medium in "Hajyusefi" cultivar, respectively (Table 3). The result of the effects of BA concentrations are shown in Table 4. The leaf number changed between 4 in "Zard" cultivar without using BA to 9 in "Hajyusefi" cultivar with application of 0.5 mgL^{-1} BA, respectively (Table 4). The highest leaf number (9) was in "Hajyusefi" cultivar in WPM medium complementd with 0.5 mgL^{-1} and the least one (2) was in "Zard" cultivar in MS medium without using BA (Table 2).

Table1. Experimental variants used in initiation phase.

Media	Woody Plant Medium	Murashig&Skoog	.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}
Benzyladenine	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

Table 2. The effects of media and BA concentrations on the survival rate in *Prunus avium* cvs. "Hajusefi" and "Zard".

Media	WPM		MS		QL	
Cultivar	"Hajyusefi"	"Zard"	"Hajyusefi"	"Zard"	"Hajyusefi"	"Zard"
	Survival rate (%)					
0 mgL^{-1} BA	33.3d*	17 e	17 e	0 f	17 e	0 f
0.5 mgL^{-1} BA	83.3 a	50.0 c	50 c	17 e	66.6 b	33.3 d
1 mgL^{-1} BA	50.0c	33.3 d	33.3 d	0 f	33.3 d	17 e
	Necrosis rate (%)					
0 mgL^{-1} BA	66.6 c	83.3 b	83.3 b	100 a	83.3 b	66.6 c
0.5 mgL^{-1} BA	17.0 f	50.0 d	50 c	83.3 b	33.3 e	66.6 c
1 mgL^{-1} BA	50.0d	66.6 c	66.6 c	100 a	66.6 c	83.3 b
	Leaf number					
0 mgL^{-1} BA	7 e	6 f	4 h	2 i	5 g	4 h
0.5 mgL^{-1} BA	11 a	10 b	8 d	7 e	10 b	9 c
1 mgL^{-1} BA	10 b	9 c	7 e	6 f	9 c	8 d

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

Discussion

Explants were collected in spring on the basis of Tao *etal.* (1976), 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. Apex size of 0.5-0.7 mm were used on the basis of Jakab*et al.* (2008) that said application of bigger explants caused easier culture. Also, said that the existence of more nutrient materials and endo plant growth regulators maked more survival rate. The results of screening for an optimal basal medium on meristem culture of *Prunus avium* cvs. "Hajyusefi " and "Zard" are shown in fig 1. The highest survival rate of meristem tips was 55.5% on the WPM medium in "Hajyusefi" cultivar and 38.9% in "Zard" cultivar

(Fig 1). Our result showed that WPM medium (55.5-38.96) was better than QL (38.9-2.43%) and MS (33.4-5.7%) media on the survival rate of meristems in "Hajyusefi" and "Zard" cultivars, respectively (Fig 1). This result is agreemented with Sugiure *et al.* (1986) that reported 1/2 MS or WPM medium was suitable for the culture of Japanese persimmon meristems. Nitrogen concentration of WPM medium is less than that of MS medium, therefore the nitrogen level may have been excessive in MS media (Soliman, 2012). BA treatments significantly increased the survival rate compared with the untreated media (control). The using of the 0.5 mgL^{-1} BA inhanced the survival rate in both cultivars (Fig 2). The poor response of survival ability was noticed in other concentrations and different combinations of the growth regulators (Fig 2). Mean comparision of the effects of media, plant growth regulators and cultivar were significant in 5%. The most and the least survival rate observed in WPM medium supplemented with 0.5 mgL^{-1} BA in "Hajusefi" cultivar and MS medium in concentraton of 0 and 1 mgL^{-1} BA in "Zard" cultivar(83.3-0%) (Table 2). This result is agreemented by Comlekcioglu *et al.* (2007) and Salami *et al* (2005) that obtained the best result with application of 0.5 mgL^{-1} BA for fig cultivar "Bursa" and *Vitis vinifera* cv. "Bidanesefid", respectively. The results of basal medium, BA concentrations and cultivars on the necrosis rate of *Prunus avium* L. cvs. "Hajyusefi" and "Zard" were significant in 5% (Table 2). The most necrosis rate (100%) was observed in MS medium in 1 mgL^{-1} BA (Table 2). Although The reason full strength MS was not being successful for culturing cherry apices might be attributed to high concentration of nitrogen and/or high total salts (Soliman, 2012). Although application of 1 mgL^{-1} BA didn't showed differences with control and couldn't counteract with necrosis (Table 2). The lowest necrosis rate (17%) was observed in WPM medium complemented with 0.5 mgL^{-1} BA in "Hajyusefi" cultivar. This result is agreement with Mert and Soylu (2010) and Ozturk (2004) that BA levels could affect the browning ratio so that, optimum BA concentrations were 0.5 mgL^{-1}.

Table 3. The effects of media on the Leaf number in *Prunus avium* cvs. "Hajusefi" and "Zard".

Cultivar / Media	"Hajyusefi"	"Zard"
WPM	9.33 a	8.30 b
MS	6.33 d	5.00 e
QL	8.00 b	7.00 c

*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 on the Leaf number in *Prunus avium* cvs. "Hajusefi" and "Zard".

Cultiva / BA (mgL^{-1})	"Hajyusefi"	"Zard"
0 mgL^{-1} BA	5.33 d	4.00 e
0.5 mgL^{-1} BA	9.33 a	8.66 b
1 mgL^{-1} BA	9.00 ab	7.66c

*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 on the leaf number of "Hajyusefi" and "Zard" cultivar are shown in Table 3. The leaf number changed between 5 in Ms medium in "Zard" cultivar to 9 in WPM medium in "Hajyusefi" cultivar, respectively (Table 3). The result of the effects of BA concentrations on the leaf area are shown in Table 4. The leaf number changed between 4 in "Zard" cultivar without using BA to 9 in "Hajyusefi" cultivar with application of 0.5 mgL^{-1} BA, respectively (Table 4). The highest leaf number (9)

was in "Hajyusefi" cultivar in WPM medium complementd with 0.5 mgL^{-1} and the least one (2) was in "Zard" cultivar in MS medium without using BA (Table 2). This result is agreemented with Pruski *et al* (2000) and Ďurkovič (2006) reported that small curly shoots or poor leaf expansion on media induced with using higher concentration of BA.

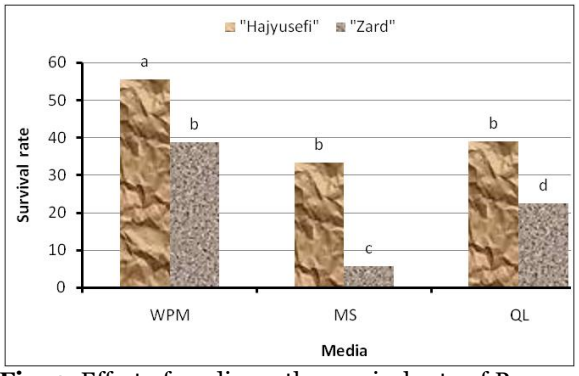

Fig. 1. Effect of media on the survival rate of Prunus avium Cvs. "Hajusefi" and "Zard".

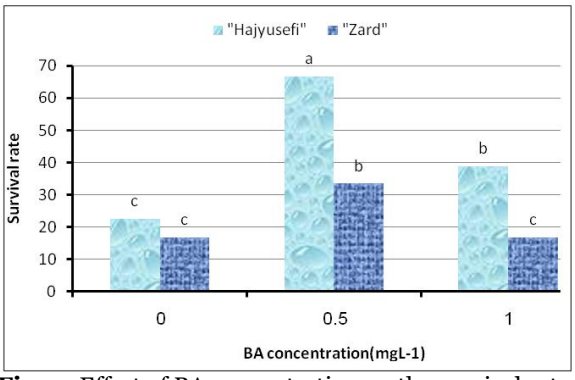

Fig. 2. Effect of BA concentration on the survival rate of Prunus avium Cvs. "Hajusefi" and "Zard".

Abbrevation

6- Benzyladenine : BA.

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