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***Research Article***

# THE EFFECTS Of MEDIA, PLANT GROWTH REGULATORS AND APEX SIZE ON THE SUCCESS of MERISTEM CULTURE IN *PRUNUS AVIUM* CV PISHRAS-E-MASHHAD

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## ABSTRACT
The aim of this study was to determine of the best size apex, media and plant growth regulators concentrations on the initiation of meristem tips from Sweet cherry trees cv. "Pishras-e- Mashhad". Ethanol and NaOCl were applied for disinfection. 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. Two apex sizes (0.2-0.4 and 0.5-0.7 mm) were used. Shoot tips were soaked in antioxidant solutions (100 $mgL^{-1}$ ascorbic acid and 150 $mgL^{-1}$ citric acid for 1 hours). Result showed that the highest survival rate and the low necrosis rate were in WPM medium supplemented with 1 $mgL^{-1}$ BA (83.3%). The using of apex size in 0.5-0.7 mm and antioxidant solutions increased survival rate in meristems. Necrosis and contamination rate was high in MS (66.6%) and QL (36.1%) media without application of BA concentration, respectively.
***Keywords:*** *Sweet Cherry, Meristem Culture, Initiation Stage, Survival Rate, Necrosis Rate*
**Abbrevation:** 6- Benzyladenine : BA

## INTRODUCTION
Sweet cherry is one of the world's important and attractive fruits. Many stone fruits like sweet cherry (*Prunus avium* L.) have been cultivated since ancient times (Naderiboldaji *et al*., 2008). 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 Iran, sweet cherry cv. "Pishras-e- Mashhad" is valuable due to its good taste, short ripening period, and the fact that it blooms in the spring, the first season of the year (Ganji and Bouzari, 2009). It is well known the fact that the propagation of woody fruit species and in general that of stone fruit species is difficult through tissue cultures and especially the propagation systems through meristems (Jakab *et al.*, 2008). Shoot tips and axillary shoots are easier to propagate compared with other types of explants such as meristems (Cassells, 1991; Gholamhoseinpour *et al*., 2012). Meristem culture is often used to produce pathogen free plants from a systemically infected individual and is also recommended for establishing aseptic explants for micropropagation. In woody species and particularly in *Prunus*, the available reports on meristem tip culture are rather limited (Manganaris *et al.*, 2003; Pérez-Tornero and Burgos, 2000). The regeneration through meristem culture is an advanced biotechnological technique 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 it occupies a central place (Jakab *et al*., 2008). The cutting season and time of the explants from the stocks are also important factors. 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). This situation can affect the browning

of the explants seen in the woody plants. 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 (Golaśin and Radojević, 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 (Dobránszki *et al*., 2000; Radmann *et al*., 2002; Chakrabarty *et al*., 2003). 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 favorite 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.
The aim of the present study was to investigation of possibility producing *of plantlets in Prunus avium* cv. "Pishras-e- Mashhad"by meristem culture.

## MATERIALS AND METHODS

### *Plant Material and Explants Preparation*

Shoot tip explants were taken from mature Sweet cherry trees cv. "Pishras-e- Mashhad" 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 with70% 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 0.2-0.4 and 0.5-0.7 mm tall (Table 1).

### *Screening of Basal Medium for Meristem Tip Culture*

Three media were used for meristem culture: MS (Murashig and Skoog, 1962), WPM (Lioyd and Mccown, 1998) 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 $mgL^{-1}$), 1$mgL^{-1}$ 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 were determined.

**Table1: Experimental variants used in initiation phase**

| **Media** | **Woody Plant Medium** | | | **Murashige & Skoog** | | | **Quoirin & Lepoivre** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Apex size A 0.2-0.4 mm** | | **Apex size B 0.5-0.7 mm** | **Apex size A 0.2-0.4 mm** | | **Apex size B 0.5-0.7 mm** | **Apex size A 0.2-0.4 mm** | | **Apex size B 0.5-0.7 mm** |
| **BA treatments** | **0 mgL$^{-1}$** | **0.5 mgL$^{-1}$** | **1 mgL$^{-1}$** | **0 mgL$^{-1}$** | **0.5 mgL$^{-1}$** | **1 mgL$^{-1}$** | **0 mgL$^{-1}$** | **0.5 mgL$^{-1}$** | **1 mgL$^{-1}$** |
| Indol buteric 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$^{-1}$ | | |
| 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 | | |

***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).

## RESULTS AND DISCUSSION

Explants were collected in spring on the basis of Tao *et al.,* (1976), Das and 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* cv. "Pishras-e- Mashhad" are shown in fig 1. The highest survival rate of meristem tips was 70.3% on the WPM medium (Figure 1). Our result showed that WPM media was better than MS (44.4%) and QL (33.3%) media on the survival rate of meristems (Figure 1). This result is agreement 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. Usage of the highest concentration of BA (1 mgL$^{-1}$) enhanced the survival rate in all three media (Figure 2). The poor response of survival ability was noticed in other concentrations and different combinations of the growth regulators (Figure 2). This result is agreement with Pérez-Tornero and Burgos (2000) that highest concentration of BA (3.11 µM ) increased survival rate and also with Salami *et al.*, (2005) that *Vitis vinifera* cv. Shahrudi had the best response in 1 mgL$^{-1}$ BA. Increasing of apex size and BA concentration improved survival rate, so that application of 0.5-0.7 mm apex size and 1 mgL$^{-1}$ BA increased survival rate (77.7%) and application of 0.2-0.4 mm apex size without BA treatments (control) decreased this item to 22.23% (Figure 3). This result is agreement with Jakab *et al.*, (2008) that said application of bigger explants caused easier culture. Also, said that the existence of more nutrient materials and endow plant growth regulators make more survival rate. Mean comparison of the effects of media, plant growth regulators and apex size were significant in 5%. The most and the least survival rate observed in WPM medium supplemented with 1 mgL$^{-1}$ BA with apex size of 0.5-0.7 mm and QL medium without BA treatment with apex size of 0.2-0.4 mm, respectively(83.3-17%) (Table 2).

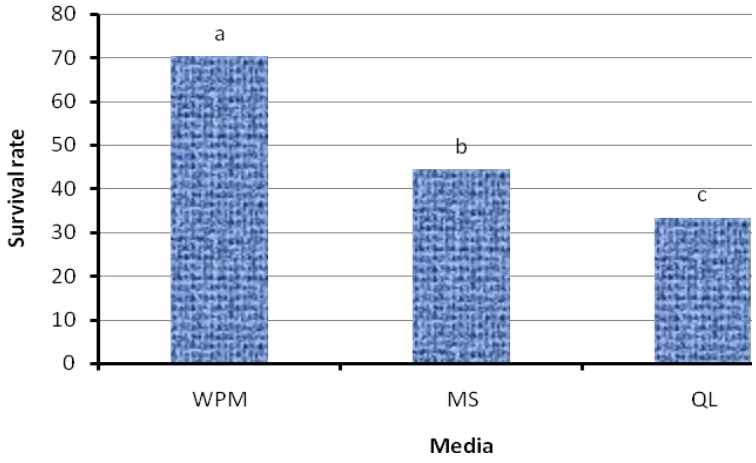


**Figure 1: Effects of media on the survival rate of *Prunus avium* cv. Pishras-e- Mashhad**

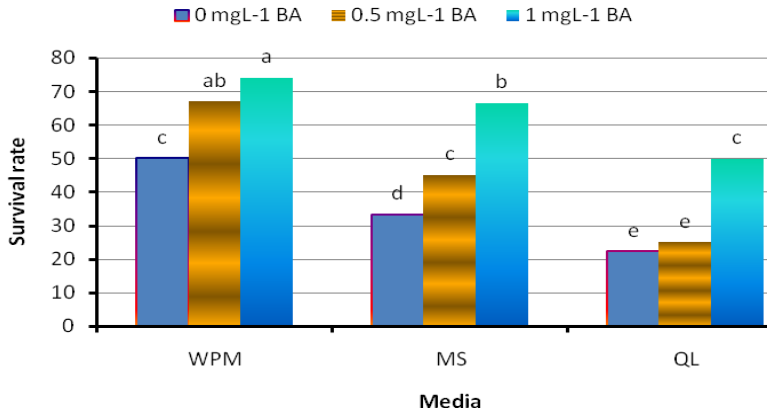


**Figure 2: Effects of Media and BA concentrations on the survival rate of *Prunus avium* cv. Pishras-e- Mashhad**

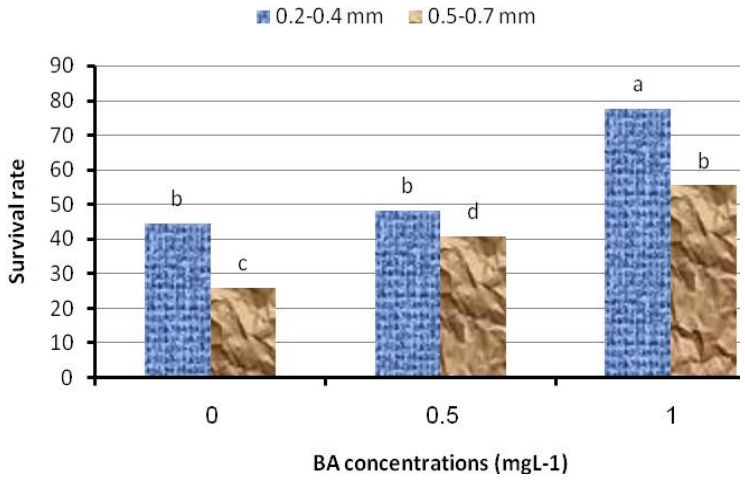


**Figure 3: Effects of apex size and BA concentrations on the survival rate of *Prunus avium* cv. Pishras-e- Mashhad**

**Table 2: The effects of media, BA concentrations and apex size on the survival rate in *Prunus avium* cv. Pishras-e- Mashhad**

| | Survival rate (%) | | | | | |
|---|---|---|---|---|---|---|
| **Media** | **WPM** | | **MS** | | **QL** | |
| **Apex size** | **A** | **B** | **A** | **B** | **A** | **B** |
| 0 $mgL^{-1}$ BA | 50 c* | 33.3 d | 33.3 d | 33.3 d | 33.3 d | 17 e |
| 0.5 $mgL^{-1}$ BA | 66.6 b | 50 c | 33.3 d | 33.3 d | 33.3 d | 17 e |
| 1 $mgL^{-1}$ BA | 83.3 a | 50 c | 50 c | 50 c | 50 c | 33.3 d |

*Means with similar letter in each column are not significantly different at 5% level by Duncan's multiple range test.*
*Apex size A: 0.2 -0.4 mm*
*Apex size B: 0.5-0.7 mm*

**Table 3: The effects of media, BA concentrations and apex size on the necrosis and contamination rate in *Prunus avium* cv. "Pishras-e- Mashhad"**

| | Necrosis rate (%) | | | | | | Contamination rate (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Media** | **WPM** | | **MS** | | **QL** | | **WPM** | | **MS** | | **QL** | |
| **Apex size** | **A** | **B** | **A** | **B** | **A** | **B** | **A** | **B** | **A** | **B** | **A** | **B** |
| 0 $mgL^{-1}$ BA | 50.0 b* | 66.6 a | 66.6 a | 66.6a | 33.3 c | 46.9 c | 0.0 d | 0.0 d | 0.0 d | 0.0 d | 33.4 b | 36.1 a |
| 0.5 $mgL^{-1}$ BA | 33.3 d | 16.6 e | 66.6 a | 66.6 a | 66.6 a | 50.0 b | 0.0 d | 16.6 c | 0.0 d | 0.0 d | 0.0 d | 33.0 b |
| 1 $mgL^{-1}$ BA | 0 f | 16.6 e | 50.0 b | 50.0 b | 50.0 b | 33.4 d | 16.6 c | 33.4 b | 0.0 d | 0.0 d | 0.0 d | 33.3 b |

*Means with similar letter in each column are not significantly different at 5% level by Duncan's multiple range test.*
*Apex size A: 0.2 -0.4 mm*
*Apex size B: 0.5-0.7 mm*

The results of basal medium, BA concentrations and apex size on the necrosis and contamination rate of *Prunus avium* L. cultivar "Pishras-e- Mashhad" were significant in 5% (Table 3). The most necrosis rate (66.6%) was observed in MS media in control (0 $mgL^{-1}$ BA). Although application of 0.5 $mgL^{-1}$ BA didn't showed differences with control and couldn't counteract with necrosis. In addition to, in MS media, there were not differences between apex sizes on the necrosis rate (Table 3). 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). The lowest necrosis rate (0%) was observed in WPM media complemented with 1 $mgL^{-1}$ BA with 0.5-0.7 mm apex size. The Using of smaller meristems (0.2-0.4 mm) increased the contamination rate in comparison with bigger meristems (Table 3). Maybe bigger meristems could absorb nutrient materials to their tissues and become more successful in remove of fungals in comparison with smaller meristems. Cassells (2001) reported that bacterial and fungal spores will grow rapidly on the rich culture medium and is agreed with this result (Table 3).

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