J. BIOL. ENVIRON. SCI.,
2015, 9(26), 57-64

Original Research Article

Regeneration of Two Sweet Cherry Cultivars cvs. "Bing" and "Dovomras" *In Vitro*

Mahbube Zamanipour[1], Ali Tehranifar[2*], Ebrahim Ganji Moghadam[3] and Bahram Abedi[4]

[1] Phd student of Horticulture, Ferdowsi University of Mashhad, International Campus, Mashhad, IRAN
[2, 4] Department of Horticulture, College of Agriculture, Ferdowsi University of Mashhad, Mashhad, IRAN
[3] Department of Horticulture, Khorasan Razavi, Agricultural and Natural Resources Research Center, Mashhad, IRAN

Received: 16.08.2015; Accepted: 08.10.2015; Published Online: 30.11.2015

ABSTRACT

In this article we will present the main aspects regarding the behavior of two Sweet cherry cultivars in the process of meristem culture *in vitro*. The effect of three growth media including WPM, MS and QL and three different hormone combinations contains BAP (0, 1 and 2 mgL^{-1}), 0.5 GA3 and 0.1 IBA were investigated on the multiplication of two Sweet cherry cultivars "Bing" and "Dovomras". 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. After six weeks, the number and length of new shoots, plantlets quality and leaf number were recorded. This experiment was arranged in a completely randomized designed. The result showed that there were significant differences between cultivars for the number and the length of shoots. Also there was negative relationship between shoot number and length. The highest average new number (5.66) of shoot multiplication and Plantlets quality was obtained in WPM supplemented with 2 mgL^{-1} BAP in "Bing" cultivar and the highest average length of shoots (2.23 cm) and leaf number induced when was cultured on MS media obtained with 1 mgL^{-1} BAP in "Dovomras" cultivar. In general, Plantlets had better quality on the WPM medium in comparison with other media. The results showed that Sweet cherry was more stable and had a higher regeneration in micropropagation process based on morphological traits.

Keywords: Meristem culture, Multiplication, Shoot number, Shoot length, Leaf number, Plantlets quality
Abbreviations: BAP: Benzyl amino purine, GA3: Gibberelic acid, IBA: Indol buteric acid

İki Kiraz Çeşidi "Bing" ve Dovomras"ın *In Vitro* Ortamda Üretimi

ÖZ

Bu çalışmada *in vitro* meristem kültürü sürecinde iki kiraz çeşidinin davranışları üzerine incelemeler yapılmıştır. Üç farklı gelişme ortamı (WPM, MS ve QL) ve üç farklı hormon [BAP (0, 1 ve 2 mgL^{-1}), 0.5 GA3 ve 0.1 IBA] kombinasyonunun iki farklı kiraz çeşidi "Bing" ve "Dovomras"ın çoğalması üzerine etkileri araştırılmıştır. Kültürler 26 C'de 16:8 (ışık:karanlık) fotoperiyotta 2000-3000 lüx ışık yoğunluğunda beyaz floresan ışık altına yetiştirilmiştir. Altı hafta sonunda yeni sürgünlerin sayısı, genç bitki kalitesi ve yaprak sayıları kaydedilmiştir. Denemeler rastgele olacak şekilde dizayn edilmiştir. Sonuçlar, sürgün sayısı ve uzunluğu bakımından çeşitler arasında farklılıkları olduğunu göstermiştir. Buna ek olarak sürgün sayısı ve uzunluğu arasında eksi yönlü ilişki tespit edilmiştir. En yüksek ortalama yeni sürgün sayısı (5.66) ve genç bitki kalitesi 2 mgL^{-1} BAP ile desteklenen WPM ortamında geliştirilen "Bing" çeşidinde elde edilirken, en yüksek ortalama sürgün uzunluğu (2.23 cm) ve yaprak sayısı 1 mgL^{-1} BAP ile birlikte MS ortamında geliştirilen "Dovomras" çeşidinde elde edilmiştir. Genel olarak genç bitkiler WPM ortamı içerisinde diğer ortamlara göre daha kaliteli olmuştur. Sonuçlar incelendiğinde morfolojik uygulamalara dayalı mikro çoğaltım süreçlerinde kirazın daha stabil ve yüksek yenilenme gücüne sahip olduğu görülmektedir.

Anahtar Kelimeler: Meristem kültürü, Çoğaltım, Sürgün uzunluğu, Yaprak sayısı, Genç Bitki Kalitesi
Kısaltmalar: BAP: Benzil Amino Pürin, GA3: Gibellerik Asit, IBA: Ondol Büterik Asit

INTRODUCTION

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 (Isac *et al.* 2010). 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 meristem culture occupies a central place (Jakab *et al*. 2008). 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 Doina *et al*. 2007). Meristem tips can easily be obtained from the actively growing shoot tips (Ozturk 2004). As this sweet cherry cultivar has excellent properties and rapid changes in the

* Corresponding author: Tehranifar@um.ac.ir

assortment on the market are trend, micropropagation is obviously an ideal method of rapid propagation of this cultivar and its subsequent introduction into production (Ruzic and Vojuvic 2008). The efficacy of shoot multiplication is influenced by several factors, such as cultivar, media composition, plant growth regulators, etc. (Dobránszki *et al*. 2010). The Shootlet formation from the meristem tips varied among cultivars. Some differences were observed, probably due to the growth characteristics of cultivars and hormonal balance within the shoots (Mert and Soylu 2010). It is well known that cytokinins promote cell division and cell expansion in plant tissue culture and many studies have reported suitable cytokinin types and their concentrations for each species. BAP could be used successfully to induce shoot multiplication in *Prunus* spp. (Pruski *et al.* 2000). Soni *et al*. (2011) found there is a relationship between BAP concentration, shoot number and shoot size. They detected that higher concentration of BAP alone may increase the shoot number while decreasing shoot length whereas low concentrations led to longer shoots. Shoot branching depends on the initiation and activity of axillary meristems, which are hormonally controlled mainly by cytokinins; however, they act in interaction with auxins even though the auxin-effect is indirect (Ward and Leyser 2004). Soliman (2012) said that the greatest number of shoots was obtained using WPM medium supplemented with 4.0 mgL^{-1} of BA. Mert and Soylu (2010) in meristem culture of apple stocks said that the most multiplication was induced in MS medium supplemented with 1 mgL^{-1} BAP , 0.5 mgL^{-1} GA3 and 0.1 mgL^{-1} IBA. Zou (2010) achieved successful multiple shoot Chinese plum (*Prunus salicina* Lindl.) with WPM medium supplemented with 0.1 mgL^{-1} IBA, 2 mgL^{-1} BAP, 0.3 mgL^{-1} KIN and 1.0 gL^{-1} casein hydrolysate. Jakab *et al.* (2008) in meritem culture of European plum (*Prunus domestica* L.) reported that the most multiplication made with application MS medium supplemented wuth 0.7 mgL^{-1} BAP.

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

In the present investigation attempts were made to standardize a reproducible protocol for *in vitro* clonal propagation of Sweet cherry (*Prunus avium* L.) cultivars cvs "Bing" and "Dovomras". When required subcultures were carried out at regular intervals for the formation of large number of shoots from a single culture. All work was done in a laminar air flow hood under sterile conditions. Three media were used for multiplication stage: MS (Murashig & Skoog 1962), WPM (Lioyd & Mccown 1980) and QL (Quoirin and Lepoivre 1977) basal salt medium. All media were supplemented with 0.1 mgL^{-1} IBA, 0.5 mgL^{-1} GA3 and various concentrations of BAP (Aldrich-Sigma company) (0, 1 and 2 mgL^{-1}), 3% sucrose and 2.8 gL^{-1} Agar-Agar (Aldrich-Sigma company) 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. After six weeks, the number and length of new shoots, leaf number and plantlets quality were recorded.

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

The results of basal media on multiplication of produced shoots from meristem culture of *Prunus avium* cvs. "Bing" and "Dovomras" are shown in Figure 1. "Bing" cultivar produced more shoot number than in comparison with Dovomras" cultivar in three media. Also WPM medium was better than MS and QL media for multiplication. Therefore, The highest shoot number induced with using of WPM medium in "Bing" cultivar and the least shoot number produced with QL medium in "Dovomras" cultivar (2.66- 0.58) (Figure 1). The effects of different concentrations of BAP on the number shoots of produced from meristem culture of *Prunus avium* cvs. "Bing" and "Dovomras" are shown in Table 2. The result showed that the number of new shoots per explant

increased with application of BAP treatments and the best result was obtained with usage of 2 mgL^{-1} BAP in "Bing" cultivar (3.33). Although, there wasn't any significant differences between concentrations of 1 and 2 mgL^{-1} BAP in "Dovomras" cultivar (Table 1). Mean comparision of the effects of media, plant growth regulators and cultivar for shoot numbers were significant in 5% (Table 2). Table 2 indicated that the maximum increase in the mean number of new shoots (5.33) obtained on WP medium containing 2 mgL^{-1} BAP in "Bing" cultivar (Table 2). The least new number (0.10) induced with QL medium containing 0 mgL^{-1} BAP in "Dovomras" cultivar (Table 2).

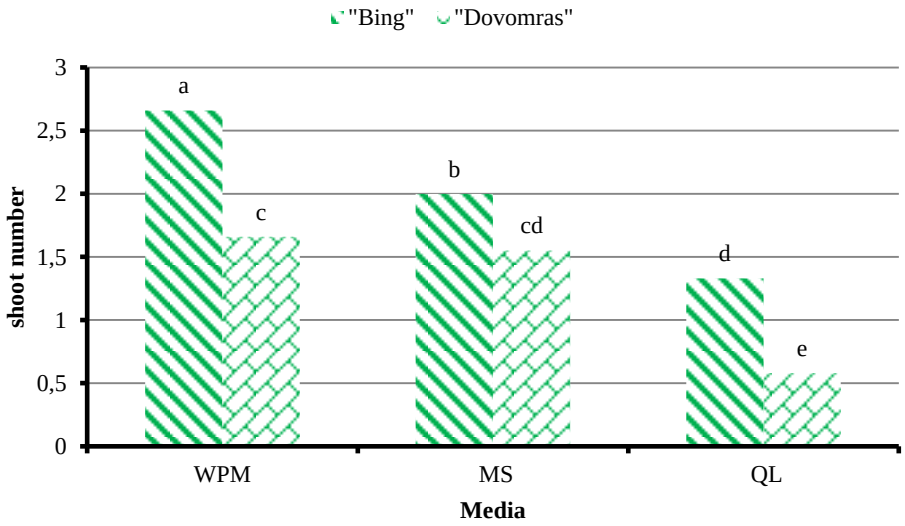

Figure 1. The effects of media on the shoot number of *Prunus avium* cvs. "Bing" and "Dovomras".

Table 1. The effects of BAP concentrations on the shoot number in *Prunus avium* cvs. "Bing" and "Dovomras".

	Cultivars	
	"Bing"	"Dovomras"
BAP (mgL^{-1})		
0 mgL^{-1}	1.00 c	0.68 d
1 mgL^{-1}	1.66 b	1.55 b
2 mgL^{-1}	3.33 a	1.56 b

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

Table 2. The effects of media and BAP concentrations on the shoot number in *Prunus avium* cvs. "Bing" and "Dovomras".

	Shoot number					
Media	WPM		MS		QL	
	"Bing"	"Dovomras"	"Bing"	"Dovomras"	"Bing"	"Dovomras"
0 mgL^{-1} BA	1.66 c	1.00 d	1.33 c	1.00 d	1.00 d	0.10 e
1 mgL^{-1} BA	3.00 b	1.66 c	2.00 c	1.66 c	1.00 d	1.00 d
2 mgL^{-1} BA	5.66 a	2.00 c	3.00 b	1.66 c	2.00 c	0.70 d

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

The results of basal media on the length of produced shoots from meristem culture of *Prunus avium* cvs. "Bing" and "Dovomras" are shown in Figure 2. The maximum increase in the mean length of shoots obtained on WPM and MS media. "Dovomras" cultivar produced more shoot length than in comparison with "Bing" cultivar in three media. Therefore, the highest shoot length induced with using of WPM and MS media in "Dovomras" cultivar and the least shoot length produced with QL medium in "Bing" cultivar (1.66- 0.70 cm) (Figure 2). Shoot length was also positively affected by the BAP concentration, and optimum results were obtained with 1mg L^{-1} of BAP in "Dovomras" cultivar (1.81 cm) (Table 3). The maximum increase in the mean

length of shoots 2.23 cm obtain on MS medium containing 1 mgL^{-1} BAP in "Dovomras" cultivar, which was the best treatments, compared the other treatments (Table 4). The results of basal media on the plantlets quality were shown in Figure 3. The most quality (2.5) induced with WPM and MS media in "Bing" cultivar and the least one made with QL medium in "Dovomras" cultivar. Generally, Plantlets had more quality in "Bing" than "Dovomras" cultivar. The mean comparison of effects BAP concentrations on the plantlets quality were shown in table 5. Cultivars had significant differences to BAP concentrations. So that, with increasing of BAP concentrations induced more plantlets quality in "Bing" cultivar. On the contrast, this item decreased with increasing of BAP concentrations in "Dovomras" cultivar. The maximum quality (2.78) induced in 2 mgL^{-1} BAP in "Bing" cultivar and the least one (1.33) produced without BAP treatments in "Dovomras" cultivar (Table 5). Mean comparison of the effects of media, plant growth regulators and cultivar for shoot numbers were significant in 5% (Table 6). The highest plantlets quality (3) produced in WPM and MS medium in "Bing" cultivar with 2 mgL^{-1} BAP and the lowest one (1) made in QL medium without BAP treatments in "Dovomras" cultivar (Table 6). The results of basal media on the leaf number were shown in Figure 4. There was a significant difference between cultivars and media for leaf number. "Dovomras" maked leaf number more than "Bing" cultivar. Also, MS medium was the best media for this characteristic. So that, the maximum leaf number (9) obtained in "Dovomras" cultivar cultured on the MS medium and the lowest number (3.64) maked in Bing" cultivar in QL medium. The mean comparison of effects BAP concentrations on the leaf number were shown in table 7. With increasing in BAP concentrations induced more leaf number in both cultivar. So that, the most leaf number (9.94) obtained in 2 mgL^{-1} BAP in "Dovomras" cultivar and the least one (2.66) was without BAP treatments in "Bing" cultivar. Mean comparison of the effects of media, plant growth regulators and cultivar for leaf number were significant in 5% (Table 8). The most leaf number (12) obtained in MS medium in "Dovomras" cultivar in 1 and 2 mgL^{-1} BAP and the least number (4) made in QL medium in "Bing" cultivar without BAP concentrations.

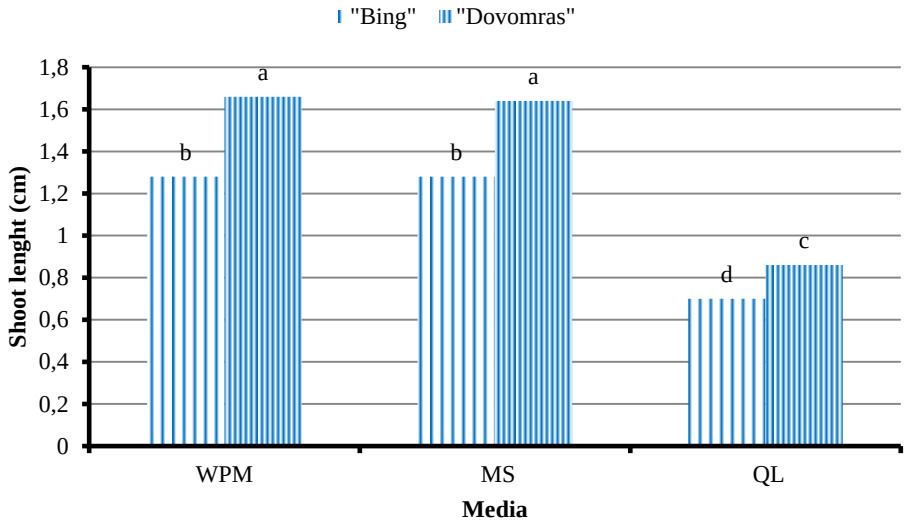

Figure 2. The effects of media on the shoot lenght of *Prunus avium* cvs. "Bing" and "Dovomras".

Table 3. The effects of BAP concentrations on the shoot lenght in *Prunus avium* cvs. "Bing" and "Dovomras".

	Cultivars	
	"Bing"	"Dovomras"
BA (mgL^{-1})		
0 mgL^{-1} BA	0.89 d	0.60 e
1 mgL^{-1} BA	1.13 c	1.81 a
2 mgL^{-1} BA	1.24 b	1.76 b

*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 shoot lenght in *Prunus avium* cvs. "Bing" and "Dovomras".

Media	Shoot length (cm)					
	WPM		MS		QL	
	"Bing"	"Dovomras"	"Bing"	"Dovomras"	"Bing"	"Dovomras"
0 mgL^{-1} BA	1.14 e	0.70 i	1.13 e	0.60 j	0.40 l	0.50 k
1 mgL^{-1} BA	1.30 d	2.20 a	1.30 d	2.23 a	0.80 h	1.00 f
2 mgL^{-1} BA	1.40 c	2.10 b	1.43 c	2.10 b	0.90 g	1.10 e

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

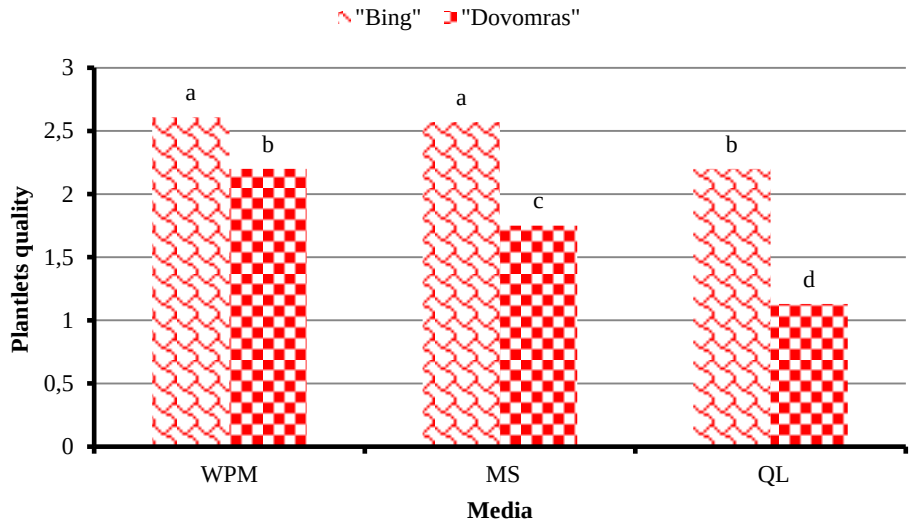

Figure 3. The effects of media on the plantlets quality of *Prunus avium* cvs. "Bing" and "Dovomras".

Table 5. The effects of BAP concentrations on the plantlets quality in *Prunus avium* cvs. "Bing" and "Dovomras".

BA (mgL^{-1})	Cultivars	
	"Bing"	"Dovomras"
0 mgL^{-1} BA	2.00 b	1.33 d
1 mgL^{-1} BA	2.60 a	2.13 b
2 mgL^{-1} BA	2.78 a	1.62 c

*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 plantlets quality in *Prunus avium* cvs. "Bing" and "Dovomras".

Media	Shoot length (cm)					
	WPM		MS		QL	
	"Bing"	"Dovomras"	"Bing"	"Dovomras"	"Bing"	"Dovomras"
0 mgL^{-1} BA	2.00 e	2.00 e	2.00 e	1.00 g	2.00 e	1.00 g
1 mgL^{-1} BA	2.83 ab	2.60 bc	2.76 ab	2.60 bc	2.20 de	1.20 g
2 mgL^{-1} BA	3.00 a	2.00 e	2.96 a	1.66 f	2.40 cd	1.20 g

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

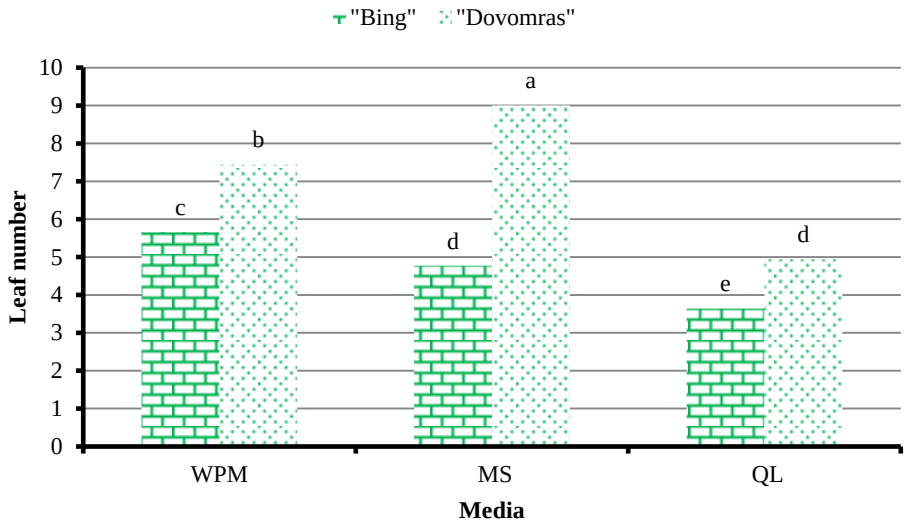

Figure 4. The effects of media on leaf number of *Prunus avium* cvs. "Bing" and "Dovomras".

Table 7. The effects of BAP concentrations on the leaf number in *Prunus avium* cvs. "Bing" and "Dovomras".

	Cultivars	
	"Bing"	"Dovomras"
BA (mgL^{-1})		
0 mgL^{-1} BA	2.66 f	3.66 e
1 mgL^{-1} BA	5.38 d	7.77 b
2 mgL^{-1} BA	6.03 c	9.94 a

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

Table 8. The effects of media and BAP concentrations on the leaf number in *Prunus avium* cvs. "Bing" and "Dovomras"

	Shoot length (cm)					
Media	WPM		MS		QL	
	"Bing"	"Dovomras"	"Bing"	"Dovomras"	"Bing"	"Dovomras"
0 mgL^{-1} BA	4.00 de	4.00 de	3.00 ef	4.00 de	1.00 f	3.00 ef
1 mgL^{-1} BA	6.00 bcd	7.33 b	5.00 bcde	11.00 a	5.16 bcde	5.00 bcde
2 mgL^{-1} BA	7.00 bc	11.00 a	6.33 bcd	12.00 a	4.76 cde	6.83 bc

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

DISCUSSION

Multiplication is a rapid increase of organs which can ultimately give rise to plant. This increase is achieved by enhancing axillary shoot initiation (Murashig, 1974). This stage is repeated at regular intervals to produce large-scale shoot multiplication to be commercially useful (Smith and Murashig, 1970). The efficacy of shoot multiplication is influenced by several factors, such as media composition, plant growth regulators, etc. (Dobránszki *et al.*, 2010). The results of basal media on the multiplication of produced shoots from meristem culture of *Prunus avium* cvs. "Bing" and "Dovomras" are shown in Figure 1. "Bing" cultivar produced more shoot number than in comparison with "Dovomras" cultivar in three media. This results is agreemented with Sedlak and Paprštein (2008) that different genotypes of sweet cherry do not respond in the same way during proliferation *in vitro* and differences in proliferation rates of two sweet cherry cultivars could result from various

auxin and cytokinin metabolism**.** WPM medium was better than MS and QL media for multiplication (Figure 1). The number of new shoots per explant increased with application of BAP treatments and the best result was obtained with usage of 2 mgL^{-1} BAP (Table 1). These results are agreemented with Zou (2012) that said the greatest number of shoots was obtained using WPM medium supplemented with 2.0 mgL^{-1} BAP and also with Comlecoglu *et al.* (2007) that application of 2 mgL^{-1} BA, 0.2 mgL^{-1} GA3 and 0.1 mgL^{-1} IBA induced the highest multiplication in fig cultivars.

The highest shoot lenght induced with using of WPM and MS media in "Dovomras" cultivar (Figure 2). Shoot length was also positively affected by the BAP concentration, and optimum results were obtained with 1mg L^{-1} of BAP in "Dovomras" cultivar (1.81cm) (Table 3). These results are agreemented with Soni *et al*. (2011) that found BAP the most effective cytokinin in shoot multiplication, and there are a relationship between BAP concentration, shoot number and shoot size. They detected that higher concentration of BAP alone may increase the shoot number while decreasing shoot length whereas low concentrations led to longer shoots. The maximum increase in the mean length of shoots 2.23 cm obtain on MS medium containing 1 mgL^{-1} BAP in "Dovomras" cultivar, which was the best treatments, compared the other treatments (Table 4). Marin *et al*. (1993) theory which describes that there is negative relationship between shoot number and length. Shoot branching depends on the initiation and activity of axillary meristems, which are hormonally controlled mainly by cytokinins; however, they act in interaction with auxins even though the auxin-effect is indirect (Ward and Leyser, 2004) that is agreemented with our results. Cultivars had significant differences to BAP concentrations. So that, with increasing of BAP concentrations induced more plantlets quality in "Bing" cultivar. On the contrast, this item decreased with increasing of BAP concentrations in "Dovomras" cultivar (Table 6). In this result distinguished that media type had significant effect on the plantlets quality. Therefore, symptoms such as leaves yellowing and death of leaves tip, vitrification and tissues browning were seen in QL medium. This result is agreemented with Perez- Torenro *et al.* (2000) that reported WPM was the best medium in meristem culture of *Prunus armeniaca* cultivars. Also, plant quality had positive relationship with multiplication. In both cultivars, With increasing in multiplication rate induced more quality.

ACKNOWLEGMENTS

This research was supported by Khorasan Razavi, Agriculture and Natural Resource Research Center, Mashhad, Iran.

REFERENCES

Clapa Doina, Al (2007). Tissue culture and ex vitro acclimation of Rhododendron sp., Buletinul USAMV-CN, nr.64.

Dobránszki J, Jaime A, and Teixeirada S (2010). Micropropagation of apple. A review. Biotechnology Advances 28: 462-488.

Ďurkovič J (2006). Rapid micropropagation of mature wild cherry. Biologia Plantarum, 50: 733–736.

Jakab IZ, Pamfil D, Clapa D, and Fira A (2008). *În vitro* regeneration and meristem culture of *Prunus domestica* cv. Bulletin UASVM. Acta Horticulturae, pp. 126-131.

Lioyd G, and 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.

Marin JA, Jones OP, and Hadlow WCC (1993). Micropropagation of columnar apple trees. Journal of Horticultural Science 68: 289.97.

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

Murashige T (1974). Plant propagation through tissue culture. Annual Review Plant Physiology 25: 135-160.

Murashige T, and 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 .

Purski KW, Lewis T, Astatkie T, and Nowak J (2000). Micropropagation of Chokecherry and Pincherry cultivars. Plant Cell, Tissue and Organ Culture, Vol. 63, pp. 93–100.

Ružić, DV, and Vujović TI (2008). The effects of cytokinin types and their concentration on *in vitro* multiplication of sweet cherry cv. Lapins (Prunus avium L.). Horticultural Science 35: 12-21.

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

Sedlák J, and Paprštein F (2008). *İn vitro* shoot proliferation of sweet cherry cultivars Karešova and Rivan. Horticultural Science 35(3): 95–98

Smith RH, and Murashige T (1970). *İn vitro* development of the isolated shoot apical meristem of angiosperms. Journal of Botany, 57: 562-568.

Snedecor GM, and Cochran WG (1986). Statistical Methods. 9 Ed., The Iowa State University, Press. Th. Amer. Iowa, U.S.A. 07 P.

Soni M, Thakur M, and Modgil M (2011). *İn vitro* multiplication of Merton I. 793-An apple rootstock suitable for replantation. Indian Journal of Biotechnology 10: 362-368.

Ward SP, and Leyser O (2004). Shoot branching. Current Opinion in Plant Biology 7: 73-78.

Zou YN (2010). Micropropagation of Chinese plum (*Prunus salicina* Lindl.) using mature stem segments. Notulae Botanica Horti Agrobotanici Cluj- Napoca, 38 (3): 214-218.