Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Archives of Applied Science Research, 2011, 3 (1):118-125

(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Effects of Growth Regulators on Micro Propagation of some Mahaleb Dwarf Genotypes *(Prunus Mahaleb L.)*

Ali Reza Daneshvar Hosseini[1], Ebrahim Ganji Moghadam[1,2], ,Saeed Kavari Khorasani[3] and Mohammad Reza Bihamta[4]

[1]Islamic Azad University,Karaj, Iran (pachenar2001@yahoo.com)
[2]Dept. of Hort. of Khorasan Razavi Agricultural Research and Natural Resource Center, Mashhad, Iran.
[3]Dept. of crop science of Khorasan Razavi Agricultural Research and Natural Resource Center, Mashhad, Mashhad, Iran.
[4]Dept. of plant breeding of Tehran university, Tehran, Iran.

ABSTRACT

Mahaleb is a shrub or tree from rosaceae family under the scientific name of "Prunus mahaleb L." which is one of the most important root stocks for cherry and sour cherry. Although the cherry root stock is not well compatible with light, calcareous and rocky ground soils and with ocean climate, but mahaleb root stock is well compatible with these soils and climate. This study was conducted to determine the best sterilization treatment, effects of plant growth regulators on micro propagation of the Mahaleb genotypes in Khorasan Razavi Agricultural Research and Natural Resource Centre. To investigate the effects of growth regulators, using 4 genotypes we sterilized the explants under the Mercuric chloride treatments. Then, we studied the different levels of two hormones of BAP and GA3 in proliferation stage and hormones of IBA and NAA in rooting stage in MS media plus 30g.l^{-1} of sucrose and 6.7g.l^{-1} of agar. The results of this study showed that the best sterilization treatment for explants is 0.1% of mercuric chloride for 2 minutes. Also, the best results for proliferation was obtained in a media containing 2 mg.l^{-1} of BAP and for the rooting stage, the best results derived from the media containing 1.5 mg.l^{-1} of IBA and 0.5% mg.l^{-1} of NAA.

Key Words: Auxin, Cytokenin, Mahaleb, Tissue culture.

INTRODUCTION

Different climates and low and high lands in Iran has been resulted in existence of land areas with very different needs (Maniei, 1993) [2]. The suitable root stocks for fruit trees and their easy and comfortable propagation has always been of importance. In other hand, due to critical role of root stock in the rate of growth, early-maturity, functionality and tolerance against the diseases, the selection of root stock will play an important role in the garden management

programs. The main problem in cherry gardens is the hyper-growth of trees. Studies show that the main rootstocks that are being used in cherry are selected from seedlings of mahaleb, sour cherries and cherries (Sansavini et al 1996) [25]. In Iran, we can point to the studies on the cherries root stocks by Izadpanah (2001) [16] on *Prunus avium* and by Goudarzi (1995) [23] on the Colt and F 12/1. Izadpanah's findings showed that WPM media (Mc Cown, et al 1980) [5] is the best media for such root stocks. In another study, Goudarzi stated that Linsmire and skoog media (1965) [9] is the best rooting media with difference in amount of sodium phosphate. Also, according to his findings, the most suitable media for rooting is the modified LS media containing 162 $mg.l^{-1}$ Phelorogelisinol. Some other studies have been done in other countries of the world as well. Results of the study of Dradi et al (1996) [11] on 11 mahaleb ecotypes as the root stock for 2 types of cherries show that the media containing SH macro elements (Shenk et al 1972) [24], micro elements of MS media(Murashig and skoog, 1962) [27], Vitamins of CH medias (Cheng, 1978) [28], 0.3 – 0.8 $mg.l^{-1}$ of BAP and 0.01 $mg.l^{-1}$ of NAA is the best proliferation media equal to 3.4. According to this recent study, the rate of proliferation is dependent on the ecotype. As a matter of fact, proliferation rate in 11 mahaleb ecotypes in the same media varied from 1.5 to 4.5. Besides, other studies showed that the percentage of rooting varied from 0 to 88 percent and this variation depends on the ecotype and the concentration of IBA that the most suitable and favorite rooting has been obtained in the range of concentration between 0.8 to 3.0 $mg.l^{-1}$ of IBA. In yet another study by Zilkah et al (1996) [26] on 3 colons resulted from intersection of Mazard kind in mahaleb, the results showed that the best proliferation media for initiation stage is the Baxus media (baxus, 1999) [22] and the best media for the proliferation of the shoot was Parfitt and Almahdi media (1994) [7]. Moreover, studies showed that the rooting explants successfully passed the compatibility stage and well grew in farm and green house. In studying the root stocks of cherries, Erbenova et al (2001) [15] observed 50% increase in the rate of proliferation in MS media containing 1.5 $mg.l^{-1}$ of BAP. In another study on the micro propagation of mahaleb root stocks (K-KK1 and S-ABI1) in in vitro conditions using the MS media, the findings of Sulusglo (2002) [21] showed that MS media contained 1 $mg.l^{-1}$ of BAP and 0.5 $mg.l^{-1}$ of IBA is the best proliferation media and MS media contained 0.5 $mg.l^{-1}$ of IBA is the most suitable rooting media. But in latter study, mahaleb root stocks encountered some problems in rooting in a way that after 7 days of darkness, the S-AB1 did not rooted. Using the explants of cherry leaf, *Prunus avium*, Lapins kinds and Sweat heart, Baghwat et al (2004) [4] stated that the percentage of proliferation is being affected by the growth regulators and the kind of the plant itself. In their study, in the optimized media which has been the media contained 2.27 or 4.54 mµ TDZ added by 0.27 mµ NAA, the proliferation rate for Lapinz kind has been 74% and for Sweet heart has been 54%. In another study on the micro propagation, Carolina et al (2006) [1] showed that the MS media containing 4.4 mµ BA, 0.49 mµ IBA and 0.29 mµ GA3 will be effective and suitable. Moreover, the best percentage of rooting obtained from 2.5 mµ IBA.

This study has been dealt with the effects of growth regulators on micro propagation of some genotypes of dwarf mahaleb which is one of the most important root stocks of cherries.

MATERIALS AND METHODS

In this study we used the explants of 4 genotypes (96, 149, 184 and 249) of Mahaleb rootstock for micro propagation between the Junes to March in Khorasan Razavi Agricultural Research and Natural Resources Center. The samples were moved from the greenhouse to the laboratory and were washed with water and dish washing liquid. Then they were divided to small pieces containing at least one bud and were moved to growth chamber.

In growth chamber, at first all explants were sterilized with ethanol 75% and then were come under the treatment of 0.1 and 0.2 % mercuric chloride for 1 and 2 minutes. After sterilization with ethanol and mercuric chloride, all the explants were washed with sterile water 3 times. Then, the explants were cultured in the prepared media. The media culture consists of MS media (Murashig and skoog, 1962) [27] containing different concentrations of cytokenins and auxins in proliferation and rooting stages. All the medias pH were adjusted on 5.7.

In initiation stage, the media was hormone-less and in proliferation stage, different concentrations of BAP hormones (0, 0.75, 1 and 2 $mg.l^{-1}$) and GA3 (0, 1, and 2 $mg.l^{-1}$) were added. In this step, after three subs culture (21 days between each subculture), the numbers and the length of the shoots were measured. . In rooting stage, IBA hormones (0, 0.5, 1.5 and 2.5 $mg.l^{-1}$) and NAA (0, 0.1, 0.5 and 1 $mg.l^{-1}$) were added and then after 45 days, the numbers and length of the root were recorded.

The cultures were grown under 16-h photoperiod, with the light intensity of 41 µmol $m^{-2}s^{-1}$ on the culture surfaces provided by cool white fluorescent tubes 40 W. The temperature was 23 ± 1^{oc} in the growth room [14].

For the sake of compatibility of rooting explants, we placed them in gifi pots contained a compound of coconut pit/ perlit in the ratio of 1 to 2. Then the explants were placed in the growth chamber for 2 weeks and then were moved to the green house.

In this study we used factorial experiment in a complete random design (CRD) with 5 replications and all the comparisons were done with Duncan multiples range test. We used SAS software for all analysis.

RESULTS AND DISCUSSION

Time of sampling

Time of sampling can be affected by the rate of pollution and the response of explants to culture. According to our observations, although the buds were active and the pollution rate was low between Junes to September, but the buds cannot be activated or they died during their movement after activation. On the other hand, the buds which were obtained on November and December were well activated and show very little pollution. The explants from other mounts do not show good response (Table 1).

The findings of this research confirm the findings of Imam (2003) [17] but it is against Ozzambak et al (1997) [10] which this difference can be attributed to the geographical conditions, media or genotype.

Tab. 1 – the comparison of the effect of the sampling times on explant activation

	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.
Active explants (%)	49	53	58	80	88	84	69	64	67
Non-active explants (%)	51	47	42	20	12	16	31	36	33

Surface Sterilization

Fungi polluting factors will appear one week after cultivation if they have not been demolish, but the bacterial factors need more times to be appeared depend on the type of the bacteria. The

pollution signs can be detected by white spots (milk white spots) on the media or on the end part of the explants.

The findings of this study showed that mercuric chloride on 0.2 % concentration for 1 minute and mercuric chloride on 0.2 % concentration for both 1 and 2 minutes have resulted in deleting the pollution. Meanwhile, the percentage of normal active explants in 0.1% concentration for 2 minutes is more effective than the ratio of normal 0.2% for both 1 and 2 minutes. Besides, due to the high concentration of chemical sterilization, this effect can miss-affects the growth of explants. So the concentration of 0.1% is more suitable. Since the time of 2 minutes is more suitable than 1 minute, ultimately the mercuric chloride on 0.2 % concentration for 2 minutes is more effective in control of pollutions. (Table 2)

Table. 2 – The comparison of the effects of sterilization treatments on the rate of pollution and life safety of mahaleb explants

Type of the treatment	Pollution (%)	Sterilization (%)	Active micro samples (%)	Non-active micro samples (%)
Mercuric chloride – 0.1 % - 1 min.	75	25	18	7
Mercuric chloride – 0.1 % - 2 min.	0	100	68	32
Mercuric chloride – 0.2 % - 1 min.	0	100	62	38
Mercuric chloride – 0.2 % - 2 min.	0	100	52	48

The best sterilization treatment was similar to the results of Kamali's study on GF677 root stocks (2001) [12], Khajedini's study on MM106 apple root stock (2001) [18] and Qavidel's study on M26 root stock (2003) [3]. This similarity was different in time of sterilization. This difference can be attributed to the size of explants, time of sampling and sterilization pre-treatments.

Explant Initiation

The prepared explants were placed in a MS media without hormones. The observations showed that the explants have been well activated and alive in this media (Fig. 1). In their studies on the propagation of Colt root stock, Goudarzi (1994) [23] and Sulsugloo (2002) [21] have determined F12/1 and mahaleb of MS medias as the best medias for this stage.

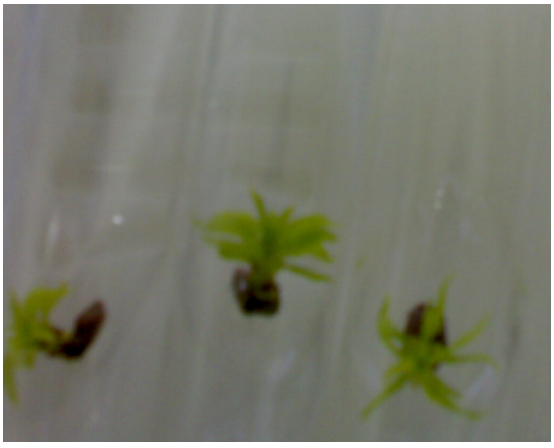

Fig.1 - Initiation and activation stage in MS media culture of Mahaleb

Proliferation

According to our observations, the media containing 2 $mg.l^{-1}$ of BAP with the average number of 3.19 shoots and 16.3 mm length will lead to the best results (Fig. 2 and 3). Erbenova et al

(2001) [15] in their study on the dwarf cherry root stocks found out that the concentration of 1.5 mg.l^{-1} will have very effective results. Moreover, Roozban et al (2002) [20] in their study on 9 kinds of pear observed that the concentration of 2 mg.l^{-1} BAP was the best treatment. These observations confirm to the findings of this study in this stage.

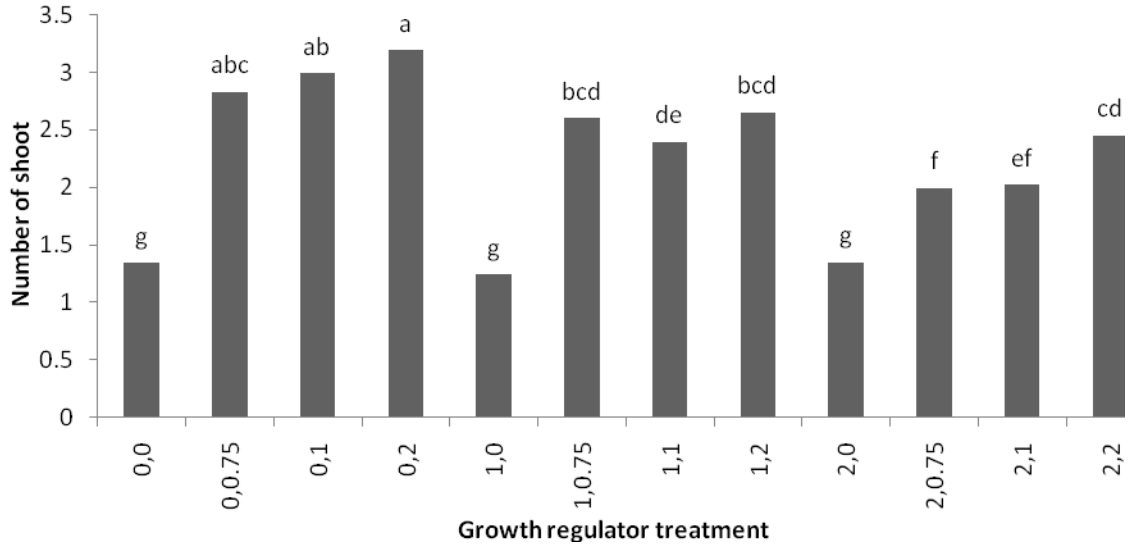

Fig.2 - The comparison of mutual effects of GA3 and BAP on the numbers of Mahaleb shoots

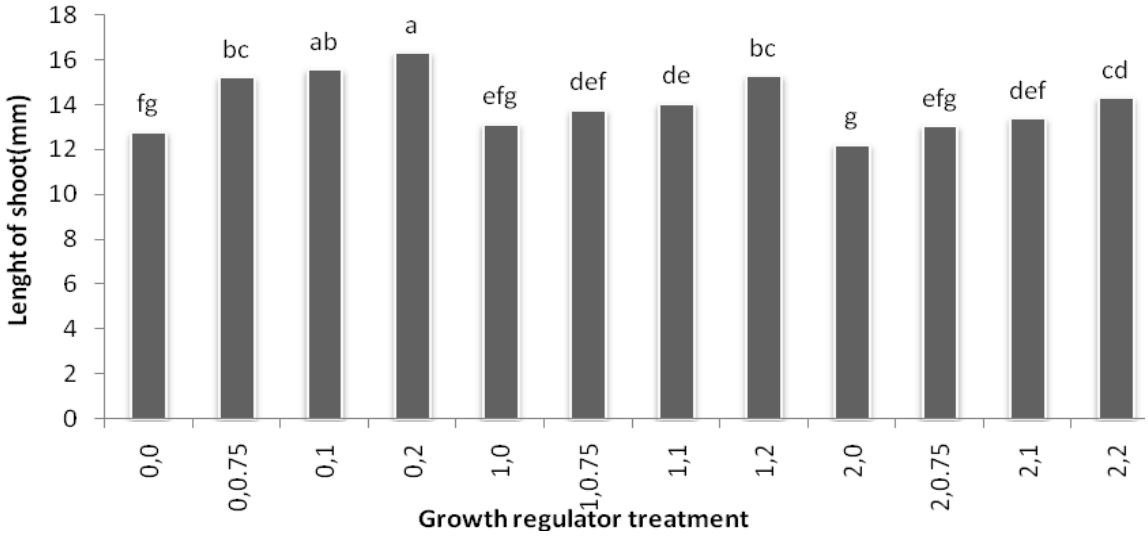

Fig. 3 - The comparison of mutual effects of GA3 and BAP on the length of Mahaleb shoots
(The numbers under the figures show the concentration of GA3 and BAP per mg.l^{-1}, respectively)

Regarding the findings of this research we believe that the presence of BAP is necessary for proliferation stage, but the GA3 has negative effects on proliferation. Finding of Ruzik et al (2008) [8] shows effectiveness of BAP rather than other hormones and the study of Wilkins et al (1982) [6] which confirms the negative effects of GA3 on the proliferation in Colt root stocks accords with our results. Besides, the reaction of the hormone can be dependent on the genotype and this fact has been confirmed in the findings of Dradi et al (1996) [11] on the mahaleb ecotypes.

Rooting

Rooting data shows that the media containing 1.5 mg.l^{-1} IBA and 0.5 mg.l^{-1} NAA is the best media for rooting on the base of the average of 3 and 3.25 numbers and lengths in comparison with others(Fig 4 and 5). Moreover, the observations show that rate of rooting varies between 40 to 87.5% (Fig. 6). So, the presence of auxin, especially IBA is effective in rooting and yet, the type of genotype can affects on selection of auxin concentration. The findings of the study of Pruski et al (2005) [13] on 2 types of cherries, the study of Dradi et al (1996) [11] on the ecotypes and Rogalsky et al (2002) [19] on 5 types of cherries genotype confirms our results.

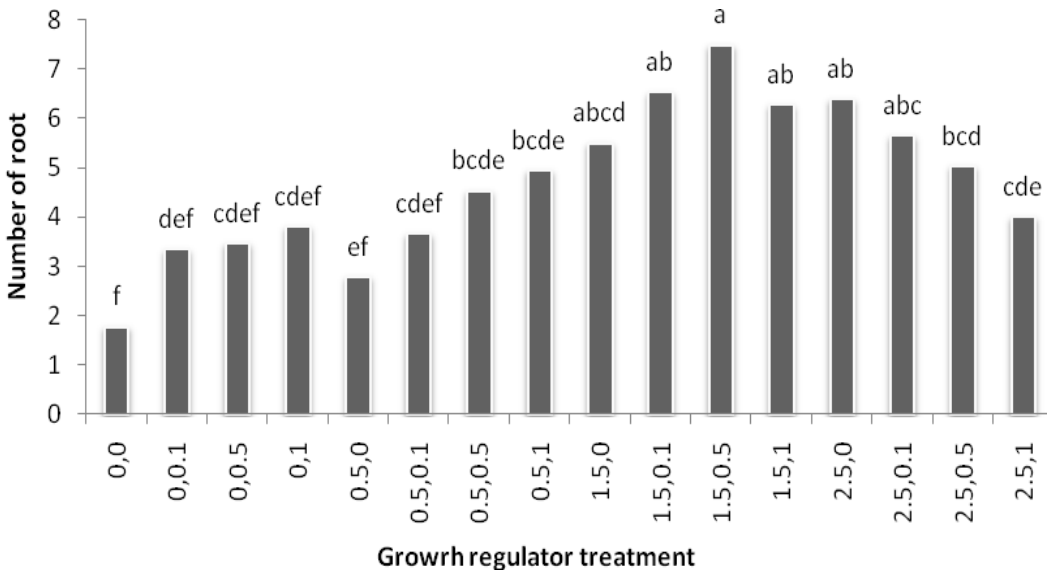

Fig. 4 - comparison of mutual effects of IBA and NAA on the roots numbers of Mahaleb.
(The numbers under the figure show the concentration of IBA and NAA per mg.l^{-1}, respectively)

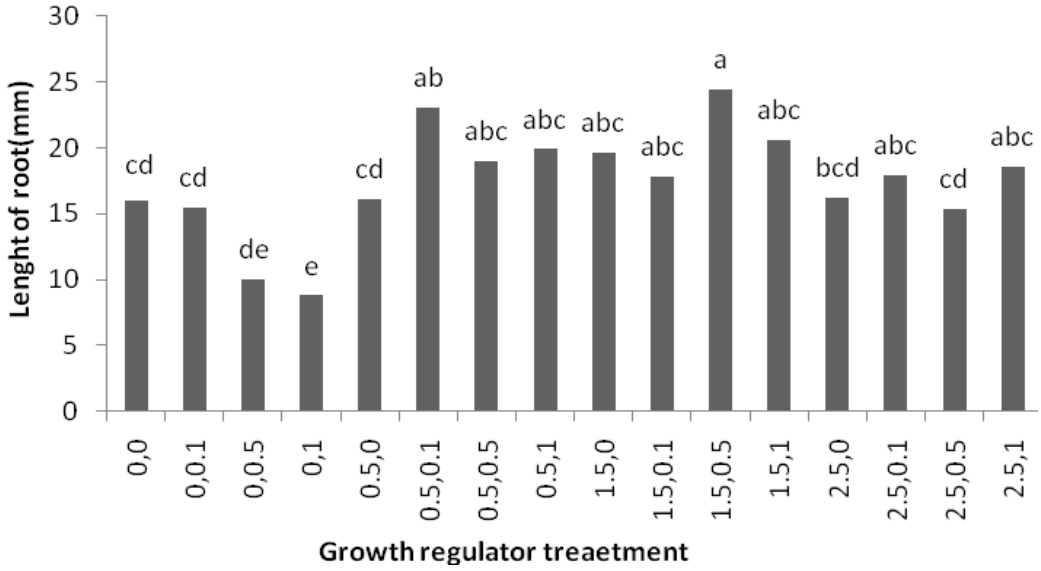

Fig. 5 - comparison of mutual effects of IBA and NAA on length of the roots of Mahaleb.

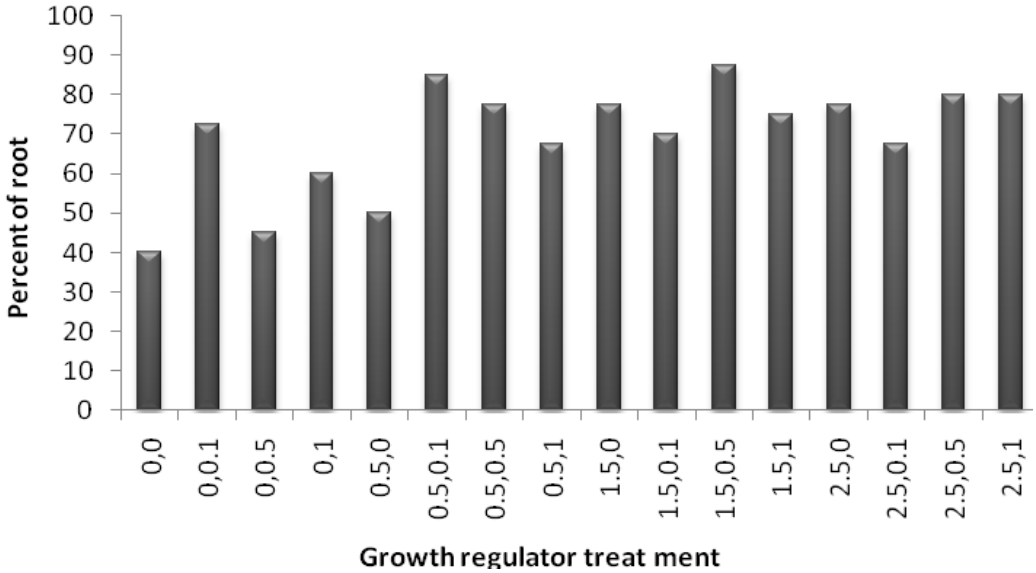

Fig. 6- comparison of mutual effects of IBA and NAA on the rate of rooting of Mahaleb.
(The numbers under the figures show the concentration of IBA & NAA per mg.l^{-1}, respectively)

Acclimization

After transfering the rooted explants to the pot, 61% of them passed the compatibility stage successfully. In this stage, the explants were irrigated with 1/2 MS media to prevent their food nutrition poverty (Fig. 7).

Fig.7. Mahaleb genotype after moving to natural conditions

CONCLUSION

The results of this research showed that we can propagate Mahaleb rootstock by in vitro method. According to this research, MS media including BAP, IBA growth regulators are most suitable for micro propagation.

Acknowledgment
We have to thank all who helped us in our laboratory experiments, in particular the personnel of Husbandry and Biotechnology Department of Khorasan Razavi Agricultural Research and Natural Resource Center, Mrs. Torabian and Mrs.Anahid.

REFERENCE

[1] A.Carolina, M.Paula, C.Pijut, H. Michler, *Horticulture science*., **2006**, 41(1),193-201.
[2] A,Maniei, Faslname *elmy va amozeshi*.,**1993**
[3] A.Qavidel. M.Sc Thesis, Islamic Azad University (Thehran.Iran, **2003**)
[4] B.Baghwat, D. Lane, Plant cell, *Tissue and Organ culture*., **2004**,78 (2),173-181.
[5] B.McCown,G.B.Lioyd, Proceedings of the intenational plant propagators society., **1980**,30,421-427.
[6] C.P.Wilkins, J.H. Dodds, Science direct., **1982**,1(3),209-216.
[7] D.E.Parfitt, A.A. Almehdi, *Can.J.Bot*., **1994**, 50:199-204**.**
[8] D.V.Ruzic, T.I. Vajovic, *Horticulture science*., **2008**, 35(1),12-21.
[9] E.M.Linsmaier, F. Skoog, *Plant cell tissue and organ culture*., **1965**,33,105-119.
[10] E.Ozzambak, S. Hepaksoy, *Acta Horticulture*., **1997**, 447,155-156.
[11] G.Dradi, G. Vito, A. stan dradi, *Acta Horticulture*., **1996**, 473-477.
[12] K.Kamali, E. Majidi, R. Zarghami, *Plant Phisiological biochemistry*.,**2001**, 175-177.
[13] K.Pruski, T. Astatkie, J. Nowak, *Plant cell, Tissue and organ culture*., **2005**, 82(2), 207-211.
[14] M.Akita, K.Negishi, A.Kitano, M.Wasaki, Y.Ohta, T.Kuriu, and T.Takii, *Acta Horticulture*., **2006**,725,579-584.
[15] M.Erbenova, F.Paprstein, J. Seldak, *Acta Horticulture*., **2001**, 560,477-480.
[16] M.Izadpanad, *Pazhohesh va Sazandegi* .,**2001**,3(14),68-74
[17] M.Imam, *Pazhohesh va Sazandegi* ., **2004,** 63 (2),10-15.
[18] M.Khajedini.M.Sc Thesis, Islamic Azad University (Thehran.Iran, **2001**)
[19] M.Rogalski, L.K. Moraeas, C. Feslibino, L. Crestani, M.P. Guerra, M.P., A.L.Silva.**,2003,** 25(2), 279-281.
[20] M.Roozban, K.Arzani, A.moeini, *Nhahal va Bazr*.,**2002**,18(3),348-361.
[21] M.Sulusoglu, Ph.D. Thesis. Ankara University(Ankara.Turkey,2002) Horticulture., **2006**, 579-584.
[22] P.Boxus, Part III.Plant propagation in vitro .Hall,R.D.(ed.) humamana press inc., Totowa NJ.111.,**1999**, 103-114.
[23] R.Goudarzi. MSc Thesis, Tarbiyatmodares university (Tehran.Iran,1995).
[24] R.U.Schenk, A.C. Hildebrandt, *Can.J.Bot*.,**1972**,50,199-204.
[25] S.Sansavinis, Lougi, S. 1996. *Acta Horticulture*.,**1996**, 410,363-371.
[26] S.Zilkah,E.Faningersh,A.Rotbaum, *Acta Horticulture*.,1996,314,516-521.
[27] T.Murashig, F. Skoog, *Physiol plant*.,**1962**,15, 473-497
[28] T.Y.Cheng, *American nurseryman*., **1978**,147,94-102.