Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Annals of Biological Research, 2010, 1 (2) :135-141
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233

Effects of Media Cultures and Plant Growth Regulators in Micro Propagation of Gisela 6 Rootstock

AliReza Daneshvar Hossini[1] , Ebrahim Ganji Moghadam[2a] and Sedighe Anahid[3]

[1]*Islamic Azad University-Karaj, Iran*
[2]*Dept. of Hort. of Khorasan Razavi Natural Resource and Agricultural Research Center, Mashhad, Iran*
[3]*Dept. of Plant Physiology of Khorasan Razavi Natural Resource and Agricultural Research Center, Mashhad, Iran*

Abstract

Gisela 6 is one of the semi-dwarf sweet cherry rootstocks which it is well adapted to many kinds of soils especially heavy ones. Keeping in view the importance of rootstock, this study was conducted to determine the best sterilization treatment, media and plant growth regulators on micro propagation of the Gisela 6 in Khorasan Razavi Natural Resource and Agricultural Research Centre. Pre-sterilized by immersion in 70% ethanol for 1 minutes. These pre-sterilized explants were then exposed to 0.1% and 0.2% mercuric chloride for 1 and 2 minutes. Results revealed that disinfesting in 0.1% mercuric chloride for 2 minutes significantly decreased contamination. Four culture media (MS, 1/2MS, LS and Modified LS) and three concentrations of BAP (0, 1, 2 mg^{-1}) plus 0.01 $mg.l^{-1}$ were applied for multiplication stage. Results showed that the best culture medium and BAP concentration are MS and 1 $mg.l^{-1}$ respectively. The mean of shoot number and length were 5.11 and 1.70 cm respectively. Four culture media (MS, 1/2MS, LS and Modified LS) and three concentrations of IBA (0, 1, 2 $mg.l^{-1}$) were applied for rooting phase. It was found, the best rooting medium was MS including 1 $mg.l^{-1}$ IBA and the percentage of rooted plantlets was 92. The mean of root number and length were 3 and 3.25 cm respectively. The survival percent under in vivo condition was 76.

Key word: Cherry, Proliferation, Rooting, IBA, BAP

INTRODUCTION

In Iran seedling *Prunus mahaleb* rootstocks used for cherry production. However, it has many disadvantages. Mahaleb trees do not grow satisfactorily in heavy, wet soils and grafted varieties are too large, fruit set is too late, harvest costs are too high and trees on Mahaleb are relatively short-lived [5,8]. In Iran Gisela 6 rootstock is one of the usable rootstocks for sweet and sour

cherries and is effective in size-controlling, grow satisfactorily in heavy, wet soils, early and good cropping.

The micropropagation is used as a useful method for propagation of vegetative rootstocks [2,19]. There is no universal medium for in vitro culture, since plant species and cultivars are genetically specific with regard to different components of the medium, which include not only organic substances, but also mineral elements (17). Erbenova et al (2001) [6] has reported 50% increase of multiplication rate on the dwarf rootstocks of sweet cherries in MS media culture containing 1.5mg.l^{-1}BAP. Ruzic, et al. (2000) [15] reported that the MS and 2MS (double macro-salts) media culture containing 4.4 mµ BA, 0.5 mµ NAA and 0.3 mµ GA3 are a suitable media culture for propagation of Gisela 5. In a study on micropropagation of *prunus avium*, the combination of 0.5 mg.l^{-1} BAP and 0.05 mg.l^{-1} TDZ are suitable for proliferation and a media culture containing 0.3 mg.l^{-1} IBA is desirable for the rooting [4]. Carolina et al (2006) [3] cultured nodal section of *prunus serotina* in MS media culture supplement with 4.44mµ BA, 0.49 mµ IBA and 0.29 mµ GA3. They also recognized the 2.5 mµ IBA as the best percentage for rooting. Akita et al (2006) [1] in micropropagation Yedoensis Matsum ×Prunus Cerasus showed the best proliferation on the MS media culture containing 30 g.l^{-1} sucrose, 0.5 mg.l^{-1} BA, 3 mg.l^{-1} GA3 and 0.1 mg.l^{-1} IBA.

Due to the importance of Gisela 6 rootstock in size controlling growth of cherry trees, in this study we investigate effects of media cultures and plant growth regulators in micropropagation of this rootstock.

MATERIAL AND METHODS

Plant material

The explants were taken from shoots of Gisela 6 rootstock maintained in the experimental greenhouse of Khorasan Razavi Natural Resource and Agricultural Research Center from June to March. Shoots were packed in plastic packages and were transferred to the laboratory. First of all, the explants were washed by water and dishwashing liquid to removed surface contamination. Then they were divided to some parts containing one bud and were pre-sterilized by immersion in 70% ethanol for 60 sec followed by a rinse with sterile distilled water. These pre-sterilized explants were then exposed to 0.1% and 0.2% mercuric chloride for 1 and 2 minutes (the results of sodium and calcium hypochloride treatments weren't suitable because of high contamination). After being sterilized by ethanol and mercuric chloride, explants were washed 3 times with sterile distilled water and then culture in medium.

Media

The cultures were grown on MS medium [14], MS 1/2, LS [13] and Modify LS in the presence of IBA and BAP growth regulators.

In the stage of proliferation, three levels of BAP (0, 1 and 2 mg.l^{-1} plus 0.01 mg.l^{-1} of IBA) were added. In this step, after three sub culture (21 days between each subculture), the numbers and the length of the shoots were measured.

In the rooting phase, the IBA in three levels (0, 1 and 2 mg.l^{-1}) were added to the above mentioned media cultures and then after 45 days, the numbers and length of the root were recorded.

Prior to autoclaving, the pH of all the media was adjusted to 5.75 with 0.1 N KOH. The media were sterilized in an autoclave for 20 min at 120 ◦C.

Culturing conditions

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±1º in the growth room [1].

The experiments were set up in a completely randomized design and repeated three times. Each treatment included five replicates (with five explants in each 250 cm3 jar and four jars in each replicate). Analysis of variance was performed on the data, and significant differences among treatment means calculated by LSD test at $P < 0.05$.

RESULTS AND DISCUSSION

Surface sterilization:

The best result was achieved from the sterilization of the explants to 0.1% mercuric chloride for 2 minutes. The results showed that among the sterilization treatments, 0.1% mercuric chloride for 1 minutes has had the highest contamination, and among other treatments, although the percentage of the contamination had been 0 but 0.2% mercuric chloride for 2 minutes has had the least explants of active sterile (table 1).

Table 1.The effects of surface sterilization treatments on contamination of explants

Treatments	Infection (%)	Active Explants (%)	Un-active Explants (%)
Mercuric chloride, 0.1%; 1 Min	85	12	3
Mercuric chloride,0.1%; 2 Min	0	73	27
Mercuric chloride, 0.2%; 1 Min	0	69	31
Mercuricchloride,0.2%; 2 Min	0	63	37

The suitable sterilization treatment resembles the findings of Imam (2004) [11] and Kamali (2001) [12] with just some little differences in the sterilization time, that can correlates such little differences to the size of explants, the time of their sampling and their relevant sterilization pre-treatments. In his study, Imam (2004) [11] detected the effectiveness of 0.1% mercuric chloride for 1.5 minutes in sterilization of buds of Iranian walnut (*Juglans regia*). Kamali (2001) [12] used the 0.1% mercuric chloride for 6 minutes in the explants sterilization of GF677 rootstocks and he gained favorite results.

Proliferation:

The proliferation results showed that the MS media culture supplement with 1 $mg.l^{-1}$ BAP with the mean of 5.11 numbers of shoot (Fig. 1) and the mean of 1.7 cm shoot length is the suitable treatment (Fig. 2 and 3).

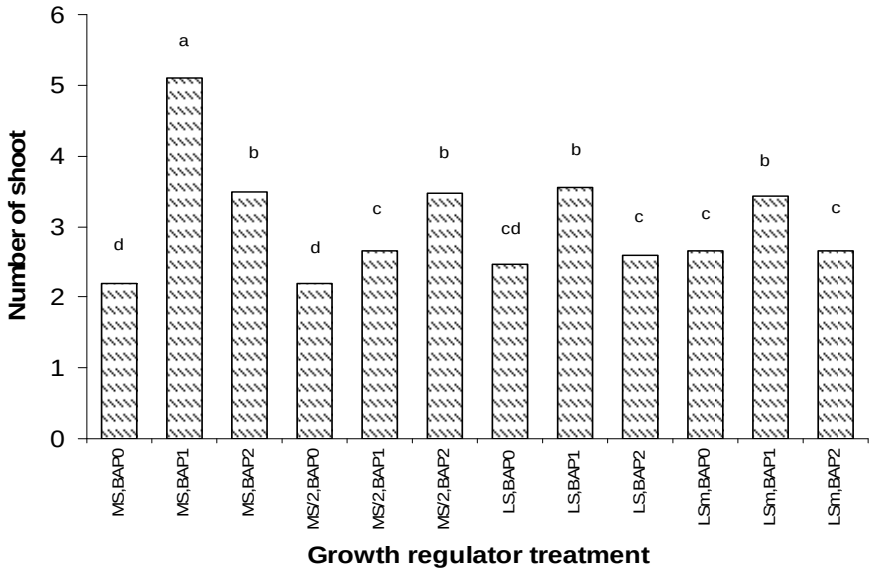

Fig. 1- The effects of different media and BAP concentrations on shoot number

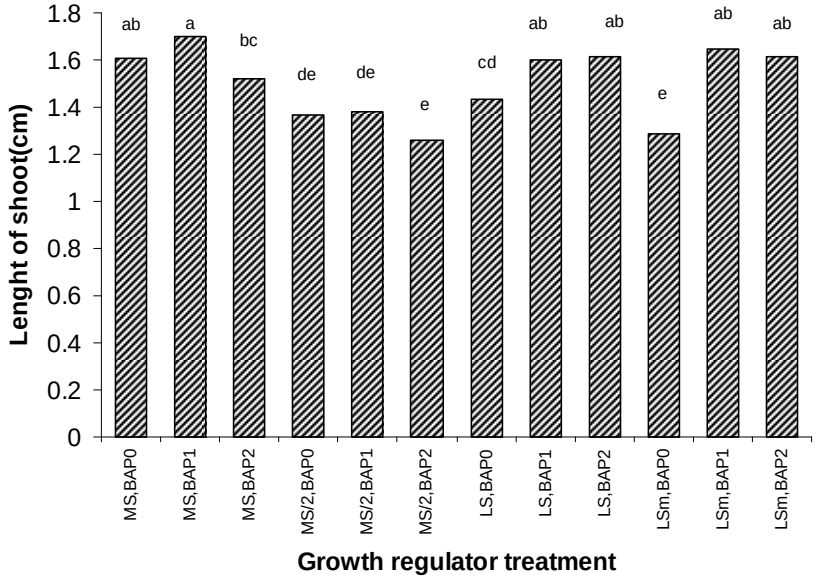

Fig. 2- The effects of different media and BAP concentrations on the length of shoot

Moreover, the considerations showed that the MS and 1/2 MS without the growth regulator has resulted in the least numbers of the shoot (2.20) and the 1/2 MS media culture containing 2 mg.l^{-1} BAP could result in the mean of 1.26 cm of shoot length (Fig 1 and 2). Our results also showed that the presence of a cytokinin like BAP in the proliferation phase is necessary. Goudarzi (1995) [7] in proliferation of colt and F12.1 rootstocks, showed 1 mg.l^{-1} BA is the most effective treatment. Besides, Sulusoglu (2002) [18] stated that the effective BAP concentration for mahaleb rootstocks (K-KK1 and S-AB1), F12.1 and SL-64 are 1 mg .l^{-1}. Jiang (2003) [9] who recognized the modify MS media culture containing 0.5 mg.l^{-1} BA and 0.1 mg.l^{-1} IBA as the most effective media culture for multiple shoot of Gisela 5 and 6 root stocks. These findings are confirmed our results.

Fig.3-The effects of media culture on proliferation and rooting of Gisela 6

Rooting:

The best root formation was observed on MS media containing 1 mg.l^{-1} IBA which produced roots readily with 92% efficiency comparison with other rooting media cultures.

The mean of numbers and length of roots in this media culture were 3 and 3.25 cm respectively (table 2 and Fig.3). The results showed that the LS media culture without growth regulators with the mean of 0.9 root numbers and the 1/2 MS media culture supplement with 1 mg.l^{-1} IBA with the mean of 0.87 cm root length had the least number and length of root respectively. Hossain et al (2003) [10] on the micropropagation of the plum reported that 1 mg.l^{-1} IBA is suitable for plum rooting and Goudarzi (1995) [7] stated that the IBA concentration for colt and F12.1 rootstoks is 1 mg.l^{-1}. These results confirm the findings of our study. In the other hand, the findings of Huang (2003) [9] were different from ours since he had recognized 0.3 mg.l^{-1} IBA as effective IBA. We can refer to the differences of genotype, the size of explants and the time of sampling as the explanation for such resulting differences.

Table 2. The Effects of different media and IBA concentrations on rooting parameters of Gisela 6

Treatments	Number of Roots	Length of Root (cm)	Rooting (%)
MS Supplemented with 0 mg .l^{-1}	2.1 b*	2.17 b	28 de
MS Supplemented with 1 mg .l^{-1}	3 a	3.25 a	92 a
MS Supplemented with 2 mg .l^{-1}	2.25 ab	1.72 c	86 ab
MS/2 Supplemented with 0 mg .l^{-1}	2.1 b	1.21 d	22 e
MS/2 Supplemented with 1 mg .l^{-1}	1.27 de	0.87 f	28 de
MS/2 Supplemented with 2 mg .l^{-1}	1.81 bc	1.03 e	48 c
LS Supplemented with 0 mg .l^{-1}	0.9 e	1.49 cd	16 f
LS Supplemented with 1 mg .l^{-1}	1.7 c	2.13 b	42 cd
LS Supplemented with 2 mg .l^{-1}	1.43 d	1.25 d	28 de
LS_m Supplemented with 0 mg .l^{-1}	2.13 b	1.08 e	32 d
LS_m Supplemented with 1 mg .l^{-1}	1.39 d	1.97 bc	66 b
LS_m Supplemented with 2 mg .l^{-1}	1.87 bc	1.33 d	58 c

Means within a column followed by the same letter are not significantly different

Acclimization:
Acclimatization was affected directly by rooting conditions. Survival was best when plantlets were transferred to pots after a short period of root emergence on rooting media. 76 % of rooted plantlets succeeded to pass the acclimization and being adapted with natural conditions (Fig.4). The results from acclimization confirmed the findings of Huang (2003) [9] and Sedlak [16] et al (2003) who reported 80 and 79.2 % of survival respectively.

Fig.4- Acclimatization of Gisela 6 rootstock on in vivo condition

CONCLUSION

The results of this research showed that we can duplicate Gisela 6 rootstock by in vitro method. According in this research, MS media including BAP and IBA growth regulators are most suitable for micro propagation.

Acknowledgment
I have to thank all who helped me in my laboratory experiments, in particular the personnel of Husbandry and Biotechnology Department of Khorasan Razavi Natural Resource and Agricultural Research Center, Eng. Mrs. Torabian. The authors are gratefull to Dr. M. Bazubandy, plant production Dept. for review the article.

REFERENCES

[1] M.Akita, K.Negishi, A.Kitano, M.Wasaki, Y.Ohta, T.Kuriu, and T.Takii, Acta Horticulture., **2006**, 579-584.
[2] G.M.Bonga, P.V.Adkares; In vitro culture of trees, 1st edition., trans. by E.Bagheri, et al., Ferdowsi University Press, Mashhad,**2004**,pp.96-104.
[3] A.Carolina, M.Paula, C.Pijut, H.Michler, *Horticulture Science*., **2006**, 41(1),193-201.
[4] J.Dorkovic, *Biologia Plantarum*., **2006**, 50 (4),733-736.
[5] M.Edin, A.Garcin, J.Lichou, JM.Jourdain, *Acta Hort*., **1996**, 410,507-510.
[6] M.Erbenova, F.Paprstein, J. Seldak, *Acta Horticulture*., **2001**, 560,477-480.
[7] R.Goudarzi. MSc Thesis, Tarbiyatmodares university (Tehran.Iran,**1995**).
[8] H.I. Hartman, D.E.Kester.1983. 4th edition., Prentice-Hall International. Inc., Englewood Cliffs, NJ,**1983**.
[9] Huang ven Jiang, *Journal of Natual Science*., **2003**, 26 (2), 161-164.

[10] S.N.Hossain, M.R.Munshi, M.R.Islam, L.Hakim, M.Hossain, *Plant Tissue Culture*.,**2003** 13 (1),81-84.
[11] M.Imam, *Pazhohesh va Sazandegi*., **2004** 63 (2),10-15.
[12] K.Kamali, A. Majidi, R. Zarqami, XI GREMPA seminar on pistachios and almonds, **2001**, 56,175-177.
[13] E.M.Linsmaier, F.Skoog, *Physiol Plant*., **1995**, 18,100-127.
[14] T.Murashige, F.Skoog, *Physiol Plant.*,**1962** 15,473-497.
[15] D.Ruzic, M.Saric, R.Cerovic and L.Culafic, *Plant Cell, Tissue and Organ Culture*., **2000**,63(1), 9-14.
[16] J.Sedlak, F.Paprstein, *Acta Horticulture*.,**2003**, 616, 379-382.
[17] M.Sarić, S.Mezei, D.Ružić. *Arch. Biol.Sci*.,**1995** 47,1-12.
[18] M. Sulusoglu, Ph.D. Thesis, Ankara University(Ankara,Turkey,**2002**).
[19] A.D.Webster, N.E.Looney. 2001. Cherries: Crop Physiology production and uses,3rd edition trans. by H. Neamati ,A. Abdollah Zadeh., Jihad Daneshgahi Press ,Mashhad,**2001**, p.51.