IN VITRO PROPAGATION OF WEIROOT 158

Mine PAKYÜREK

Siirt University, Faculty of Agriculture, Department of Horticulture, 56100, Siirt, Turkey

mine.pakyurek@siirt.edu.tr (corresponding author)

Serra HEPAKSOY

Ege University, Faculty of Agriculture, Department of Horticulture, 35100, İzmir, Turkey

serra.hepaksoy@ege.edu.tr

ABSTRACT

Clonal rootstocks are commonly preferred due to their abilities to obtain early fructification and maximum yield per unit area. *In vitro* techniques enable rapid and intensive propagation of rootstocks in laboratory conditions. In this study, propagation possibility of Weiroot 158, an important clonal rootstock used in cherry cultivation, was investigated using tissue culture technique. Shoot tips were used as explants and Murashige-Skoog (MS) was used as the nutrient media (30 g L^{-1} sucrose and 7 g L^{-1} agar). The shoot tips were placed in the temperature and light controlled culture room after planting in the nutrient medium. Samples were taken for subculture once in a month. Number of explants, leaves and sprouting were measured every 20 days. Four different MS media were tested during the shoot multiplication phase. The best shoot proliferation was obtained in a media supplemented with 4.4 µM BAP+0.49 µM IBA+0.29 µM GA_3. In addition, five different MS media were tested during the rooting phase. The results showed that the best rooting was obtained in nutrient media containing 1/2 MS + 2 mg/L IBA.

Keywords: Weiroot 158, cherry rootstock, micropropagation, plant tissue culture, plant growth regulators.

1. INTRODUCTION

Determining the appropriate rootstock and variety in the establishment of a new orchard is the most important factor affecting the increase in yield and quality in fruit production. The rootstocks in fruit growing are defined as seed and clone rootstocks according to the propagation methods. Genetic expansions, excessive vegetative growth and late yielding are the negative aspects of seed rootstocks due to the heterozygotic nature [Hartmann and Kester, 1983]. Seed rootstocks were widely used in fruit seedlings production for many years in Turkey. However, the superiority of clone rootstocks recently attracted attention; thus, the use of grafted seedlings on rootstocks has started in fruit production. Wild cherry (bird cherry) and Idris have been used as rootstocks for a long time in the cherry and sour cherry cultivation in Turkey. Wild cherry is grown in lands with fertile, deep, moist and well drained soils, while idris (*Prunus mahaleb* L.) rootstock is preferred in poor, calcareous and sloppy areas with less irrigation. In addition to seed rootstock, Gisela 5, Gisela 6, Maxma-14, Weiroot 158, Tabel, SL 64 and Mazzard semi-dwarf and dwarf rootstock clones such as F 12/1 have been brought to Turkey for modern fruit production, and studies on production opportunities of aforementioned species continue in different regions. The SL 64 for calcareous soils and Gisela 5 and Gisela 6 and Maxma-14 rootstocks for other soils are promising. Turkish Ziraat cherry, also known as 0900 variety which is well-known in the world composes a large part of the cherry production in Turkey. In addition, new cherry varieties such as Sweet Heart, Celeste, Kordia, Regina, North Wonder, Sunburst and Summit have recently been produced by farmers [Zainel and Hepaksoy, 2018].

The micropropagation techniques such as meristem, shoot tip and knot cultures are especially preferred for clonal rootstock production. The micropropagation techniques are used to obtain an exemplary plant in aseptic conditions, to produce more in a smaller area compared to the traditional method, to perform rapid propagation in a short time, to be able to produce all year round without being affected by seasonal changes and to allow easy production of difficult species [Bürün and Türkoğlu, 1996]. Basic stages of micropropagation technique are selection of plant to be explanted, taking plant samples, preparation stage including the planting of samples and preparation of nutrient media, culture stage where the explants are planted in an artificial nutrient media, propagation and development of shoots in a culture media, rooting and adaptation to the external environment [George et al., 2008].

Several *in vitro* studies have been carried out using shoots and different tissues of cherry and sour cherry which are the varieties of stone seed fruits. For example; Sauer [1985] cultivated the meristems of the top buds on young shoots of cherry rootstock, Mazzard, in MS media containing 0.1 mg L^{-1} NAA and 2 mg L^{-1} BAP and provided the development of side shoots. Similarly, Pevalek-Kozlina and Jelaska [1987] used shoot tips and side buds of wild cherry rootstock (*Prunus avium* L.) *in vitro* production. The best shoot propagation was achieved by adding 2.2 µM BA, 2.5 µM IBA and 0.3 µM GA3 to the modified WPM base nutrient media. In another *in vitro* micrografting study, Özzambak and Schmidt [1991] successfully propagated the Early Burlat and Viola cherry cultivars and F 12/1 and 209/1 rootstock shoot tips in the MS media containing 1.0 mg L^{-1} BAP. Zilkah et al [1992] studied *in vitro* production of three different clones of MxM (*Prunus avium x Prunus mahaleb*) used as cherry-sour cherry rootstock in AP (Almehdi & Parfitt) nutrient media containing 0.2 mg L^{-1} BA and 0.01 mg L^{-1} IBA. The best shoot elongation was obtained for MxM 2 clone.

The use of clone rootstocks and the production of rootstocks by tissue culture techniques should be increased in order to be able to compete with the other fruit producer countries of the world. Therefore, in this study, shoot growth and rooting performance of Weiroot 158 rootstocks in different media were investigated by using tissue culture propagation technique.

2. MATERIAL AND METHODS

2.1. Material

The study was carried out at Ege University Faculty of Agriculture, Department of Horticulture Tissue Culture Laboratory between 2007 and 2009. The shoot tips obtained from Weiroot 158 clone rootstock produced in Isparta Eğirdir Fruit Research Institute were used as explants.

The dwarf clone rootstock Weiroot 158, obtained by selection from cherries in Germany and used in cherry-cherry cultivation, is known to have a graft incompatibility with some cherry varieties, be early fruiting and be easily propagated by with green buds [Büyükyılmaz and Öz, 1994].

2.2. Method

The MS [Murashige and Skoog, 1962] nutrient media was used in this study. Indole butyric acid (IBA) and naphthalene acetic acid (NAA) from the auxin group; gibberellic acid (GA3) from the gibberellin group and 6-benzylaminopurine (BAP) from the cytokinin group were added to the MS nutrient media as plant growth regulator. In addition, 30 g L^{-1} sucrose with 7 g L^{-1} agar in the initial stage and 6 g L^{-1} agar in the rooting stage were added to the nutrient media. The pH of the nutrient media prepared with sterile purified water was adjusted using 1N sodium hydroxide (NaOH) and 1N hydrochloric acid (HCL). The nutrient media which were placed in the sterilized glass tubes or jars

were kept in an autoclave at 121 °C and 1.2 atmospheric pressure for 20 minutes and sterilized.

Four MS nutrient media in the reproduction stage and five in the rooting stage of the study were used. The names of the nutrient media used, contents of plant growth regulators, pH values and references were given in Tables 1 and 2.

2.2.1. Collection, planting and sterilization of plant samples

Shoot tip samples of plants collected in the early hours of May were wrapped in wet papers, placed in nylon bags and brought to the laboratory in the freezer to reduce water loss in the tissues. Small leaves in the plant specimens were removed carefully to prevent the damages of shoot tips and then washed with tap water to reduce the microorganism density of the material. The pre-sterilization process was completed by washing the samples from the dirt, then placing the samples in soapy water, stirring occasionally and waiting for 20 minutes, then washing under running tap water for 20 minutes. After the pre-sterilization process, samples were placed into a laminar (sterile in a horizontal and vertical plane) airflow cabinet and sterilized by keeping in 1/5 diluted solution containing 4% sodium hypochlorite for 20 minutes. Then, the sterilization process was completed by washing three times in sterile pure water for five minutes to remove the disinfectant material, and the explants were prepared for sowing into the nutrient media [Karvar and Gülşen, 1990].

2.2.2. Conditions of *in vitro* culture

The explants cultured under aseptic conditions were kept in culture chamber at 24±1 °C 16 hours light and 8 hours dark conditions during the initial, propagation and rooting stages. Moisture control was not carried out in the culture chamber since the mouths of the glass tubes used as culture containers were closed with their own lids and the mouths of the glass jars and petri dishes were covered with stretch film.

2.2.3. Experimental design and data analysis

Experiments in shoot propagation and rooting stages in petri dishes and jars were laid out according to randomized plots with three replications. Each culture plate had 5 explants. The differences in mean values of data among the nutrient media and the measurement times and the interactions between the media and the time were analyzed according to the analysis of variances (ANOVA) test by using Minitab Software. Then, the Duncan's multiple range test at 95% probability was used as post-hoc where ANOVA indicated significant differences. In statistical analysis, angle values of percentages were used.

Table 1. MS media used in the shoot propagation stage.

Name of media	Contents of plant growth regulators	pH	Reference
S1	MS + 1 mg/L BAP + 0.1 mg/L NAA + 0.1 mg/L GA_3	5.6	Ruzic et al. (1998)
S2	MS + 4.4 mM BAP + 0.49 mM IBA + 0.29 mM GA_3	5.6	Espinosa et al. (2006)
S3	MS + 1 mg/L BAP	5.6	----------
S4	MS + 1 mg/L BAP	6.2	Özzambak and Schmidt (1991)

Table 2. The MS nutrient media used in rooting stage.

Name of media	Contents of plant growth regulators	pH	Reference
K1	MS + 0.3 mg/L NAA	5.6	Kamali et al. (2001)
K2	MS + 0.3 mg/L NAA + 1.4 mg/L Thiamin	5.6	Kamali et al. (2001)
K3	MS + 0.5 mg/L IBA + 283.72 mg/L PG	5.7	Paul and Feucht (1985)
K4	MS + 0.5 mg/L NAA + 283.72 mg/L PG	5.7	Paul and Feucht (1985)
K5	1/2 MS + 2 mg/L IBA	5.6	Tang et al. (2002)

3. RESULTS

3.1. Initiation and propagation stage

The shoot tips collected at the beginning of May, when the vegetative growth continued, were sterilized and planted into four different MS nutrient media for shoot growth. After placing the rootstocks used in the study into the culture media, some of the materials were lost due to infection, tissue blackening and drying. However, since these losses were not very high to prevent the continuation of the study, experiments were carried out with the remaining healthy cultures. In addition, the glassization problem has been observed from time to time in cultures. Since this problem was not significant, no treatment has been performed. The first planted cultures (Figure 1) were subcultured with a four-week interval and replication was performed. Media experiments were started after obtaining sufficient *in vitro* shoots. In the 60 day of the experiment, shoot length, the number of leaves and number of tillerings were measured on 0 (day of experiment), 20, 40 and 60 days in micro shoots transferred to new nutrient media. The statistical analyzes and interpretations of the data were presented after the title of the relevant rootstock.

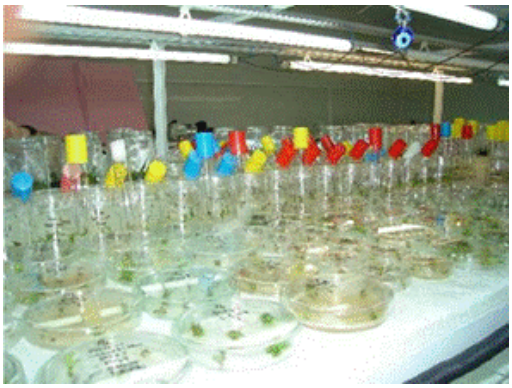

Figure 1. *In vitro* cultures during shoot propagation stage.

Sixty-day experiment of the Weiroot 158 rootstock was established in S1, S2, S3 and S4 media. Mean shoot growth value was significantly ($P<0.05$) different among the media (Table 3). The most successful shoot growth was observed in S1 (MS + 1 mg L^{-1} BAP + 0.1 mg / L NAA + 0.1 mg L^{-1}

GA3) media, followed by S2 and S4 media. The S3 (MS + 1 mg L^{-1} BAP pH = 5.6) media was the least successful media in shoot growth with a value of 14.77 mm.

Mean shoot length values in S4 and S1 media were gradually increased during the experiment. The mean shoot length in the S3 media increased until the 20th day, then decreased due to deaths in micro-shoots. In S2 medium, mean shoot length increased up to 40th day, then the mean value decreased due to drying at the ends of the shoots. The longest shoot length (28.67 mm) was obtained on day 60 in S1 medium and the shortest shoot length (13.20 mm) on day 60 in S3 medium. The mean shoot length in S4 media was 14.13 mm at the beginning of the experiment, increased to 20.80 mm after 60 days, which corresponded to a 46% increase. The mean shoot length, which was 23.13 mm at the beginning of the experiment, in S1 media reached 28.67 mm on the 60th day, which was an increase of approximately 23%. No regular increases in shoot length occurred in S2 and S3 media. The mean shoot length was 26.20 mm in S1 media, while it was 16.95 mm in S4 media.

Table 3. Mean shoot lengths of Weiroot 158 rootstock according to nutrient media.

Media	Mean shoot length (mm)				Mean
	Day 0	Day 20	Day 40	Day 60	
S1	23.13	25.73	27.27	28.67	26.20 a
S2	20.33	25.2	29.27	22.6	24.35 a
S3	14.93	16.47	14.47	13.2	14.77 b
S4	14.13	15.07	17.8	20.8	16.95 b

Mean leaf growth in micro shoots of Weiroot 158 rootstock did not show a significant difference among media, time, and media x time interaction (P> 0.05) (Table 4).

Table 4. Mean leaf numbers of Weiroot 158 rootstocks according to nutrient media.

Media	Mean number of leaves (unit/explant)			
	Day 0	Day 20	Day 40	Day 60
S1	10.33	12.07	13.47	14.60
S2	10.80	13.53	16.13	12.87
S3	9.27	10.00	9.07	8.53
S4	9.00	11.67	13.13	14.67

Similar to the shoot length, the mean number of leaves in S4 and S1 media showed a steady increase throughout the experiment. The mean value in the S3 environment increased until the 20th day, then the mean value decreased due to the drying of shoot tips. The mean number of leaves in the S2 media increased up to day 40, then the mean value decreased due to the death of micro shoots. The highest number of leaves (14.67 unit/explant) was obtained on day 60 in S4 media and the minimum leaf

number (8.53 unit/explant) was on day 60 in S3 media.

The effect of time and media on mean number of tillering was statistically significant ($P<0.05$) (Table 5). The Weiroot 158 rootstocks provided regular tillering throughout the experiment. In the first 20 days, the propagation of rootstock showed an increase of 0.42 unit/explant, while the propagation was 1.32 units/explant at day 60, which corresponded to 3-fold increase. The best proliferating media was S2, followed by S1 and S4 respectively. The least shoot growth was occurred in S3 medium.

Table 5. Mean number of tillering in Weiroot 158 rootstock.

Media	Mean number of tillering (piece/explant)			Mean
	20. day	40. day	60. day	
S1	0.33	1.27	1.40	1.00 b
S2	1.33	2.53	2.00	1.95 a
S3	0.00	0.13	0.13	0.08 c
S4	0.00	0.00	1.73	0.57 bc

The highest mean shoot length and the number of leaves were obtained from S4, S1, S2 and S3 media, respectively. The best shoot growth environments were S1, S4, S3 and S2, respectively. The mean number of tillering in S2, S1, S4 and S3 media was 1.95, 1.00, 0.57 and 0.08 unit/explant, respectively. Evaluation of these three parameters indicated that shoot growth and development of Weiroot 158 rootstock in S4 and S1 media were better compared to the S2 and S3 media. The difference between the growth status at the 5th week was shown in Figure 2.

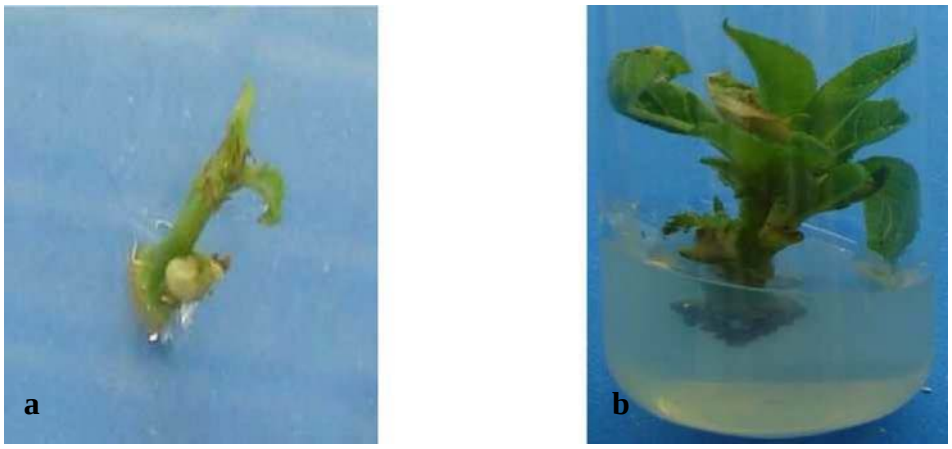

Figure 2. Weiroot 158 growth in S3 (a) and S1 (b) media at the end of the 5th week.

3.2. Rooting Stage

Five MS (Murashige and Skoog) nutrient media with different plant growth regulator contents were used for rooting micro shoots belonged to Weiroot 158 clone rootstock (Table 2). The mean rooting ratios obtained in five media were given in Table 6.

Table 6. Mean rooting ratios of Weiroot 158 rootstocks in nutrient media.

Media	Contents of Plant Growth Regulator	pH	Rooting Ratio (%)
K1	MS + 0.3 mg/L NAA	5.6	27
K2	MS + 0.3 mg/L NAA + 1.4 mg/L Thiamin	5.6	53
K3	MS + 0.5 mg/L IBA + 283.72 mg/L PG	5.7	60
K4	MS + 0.5 mg/L NAA + 283.72 mg/L PG	5.7	20
K5	1/2 MS + 2 mg/L IBA	5.6	73

The first root formation of Weiroot 158 micro-shoots planted in the rooting media was observed on the 5th day. Rooting occurred on days 7, 10, 12 and 15, respectively. The last root formation was observed on day 20. No rooting occurred after the 20th day. The plants with no roots either dried up or maintained the same developmental course. The K5 media provided the best rooting ratio (73%) for this rootstock, followed by K3 (60%), K2 (53%) and K1 (27%), respectively. The K4 medium had the lowest rooting ratio (20%). The image of a rooted Weiroot 158 rootstock in K5 (1/2 MS + 2 mg L^{-1} IBA pH = 5.6) was shown in Figure 3.

Figure 3. Weiroot 158 rootstock rooted in K5 media.

Plantlets, rooted under aseptic conditions, were carefully removed from the nutrient media without damaging the roots and the agar residues on the roots were washed thoroughly in the tap water. The plants were then immersed in 80% Pomarsol (Thiram) solution to prevent any fungi or bacterial contamination and transferred to vials containing different pre-sterilized growing media (Figure 4).

Figure 4. Rooted *in vitro* plantlets transferred to viols.

Perlite, peat, equally mixed perlite-peat, an equal amount of sand, garden soil and fertilizer mixture were added to the vials as the growth environment. Following the irrigation, the vials with plantlets rooted *in-vitro* were covered with plastic cover and placed in the climate chamber after to protect the existing moisture.

4. DISCUSSION AND CONCLUSION

Shoot tip explants were cultured in four MS nutrient media with different plant growth regulator contents to determine optimal growth conditions by tissue culture of Weiroot 158 clone rootstock. The results obtained in the propagation and rooting stages of nutrient media were different. The S1, S2, S3 and S4 media were tested during the shoot growth stage. The S4 and S1 media were more successful in shoot growth and development of Weiroot 158 rootstock compared to the S2 and S3 media.

The S1 media (1 mg L^{-1} BAP + 0.1 mg L^{-1} NAA + 0.1 mg L^{-1} GA3) contains plant growth regulators from all groups, including cytokinin (BAP), auxin (NAA) and gibberellin (GA3). The S1 media was used to investigate the effects of auxin on propagation. The results indicated that Weiroot 158 rootstock propagated well in the S1 media. Similarly, Ruzic et al. (1998) reported that cherry rootstocks provided the best growth in an MS medium with the same plant growth regulator content as in S1 media. In another study Ruzic et al. [2000] used Gisela 5 rootstock, increased the macronutrients in the ratio of 1/2 and 1/4, added 4.4 µM BA + 0.5 µM NAA + 0.3 µM GA3 and set pH to 5.78. The best growth and development occurred in the media where the macro elements were increased. In parallel with our study, Arıcı [2008] investigated the propagation possibilities of Maxma-14 and GN rootstocks used for stone seed fruit species with shoot tips and side shoots. Shoot growth for Maxma-14 and GN rootstocks was reported to be successful in media containing 2.0 mg L^{-1} BAP + 0.2 mg L^{-1} NAA + 0.5 mg L^{-1} GA3, and 1.0 mg L^{-1} BAP + 0.02 mg L^{-1} NAA + 0.5 mg L^{-1} GA3, respectively.

Shoot growth of Weiroot 158 was not very satisfactory in S2 media (4.4 µM BAP + 0.49 µM IBA + 0.29 µM GA3). Pakyürek and Hepaksoy [2019] investigated the effect of a similar media containing Floroglycinol in addition to the same plant growth regulators on shoot growth of PG using different rootstocks. Marianna GF 8/1 rootstock showed good shoot growth in the PG containing media, however, Myrobolan B and St Julien A rootstocks did not grow well in the same media. The results suggested that the addition of PG to the media may encourage shoot growth of Weiroot 158 rootstocks. In another study using a media containing the same plant growth regulators as in S2 media, Espinosa et al. [2006] investigated the shoot proliferation of Black Cherry (*Prunus serotina*). Shoot

proliferation was successfully achieved by the addition of 4.44 MSM BA, 0.49 µM IBA and 0.29 GAM GA3 into the MS nutrient media.

The S3 (1 mg L^{-1} BAP pH = 5.6) and S4 (1 mg L^{-1} BAP pH = 6.2) media contain the same growth regulator, but differ from each other only for the pH values. The S3 media was tested as an alternative to S4 media to investigate the effect of different pH levels on shoot growth. The results showed that high pH (S4) caused an increase in the shoot growth of Weiroot 158 rootstock. In contrast, the shoot growth of Weiroot 158 rootstock was quite unsatisfactory in S3 media. Similar to the S3 media used in our study, Gürel and Gülşen [1998] investigated the effects of different sucrose, agar and pH levels on shoot growth and development of Texas and Nonpareil almond (*Amygdalus communis* L.) varieties in the shoot tip study. The propagation and the best shoot growth were achieved with a sucrose dose of 5-6% and pH value of 5.5 in the first planting stage. Similarly, the best shoot growth in the sub-culturing stage was obtained in media with a pH of 5.5 and 5-6% sucrose dose. In an experiment conducted using a media similar to the S4, Aka-Kaçar et al. [2001] studied the effects of different solidifying agents and different pH levels added to the nutrient media on shoot growth, length and weight of Damil, Edabriz, Gisela 5 and MaxMa cherry rootstocks. The best results were obtained with a pH of 6.2 and agar was determined as the best solidifying agent [Aka-Kaçar et al., 2001].

The most successful rooting (73%) was achieved with K5 media containing 2 mg L-1 IBA as auxin in addition to applied 1/2 strength MS. The K5 media was followed by K3 (60%), K2 (53%) and K1 (27%). In a similar study, Al-Sabbagh et al. [1999] confirmed that successful rooting took place in IBA or NAA-containing media. Researchers who added 0.49 µM NAA or 0.49-2.45 µM IBA to the solid and liquid MS media reported the rooting after 4 weeks. Epstein et al. [1993] investigated the transport and metabolism of IBA under aseptic conditions in a study on the cherry varieties with different rooting characteristics. The 2nd, 3rd, 4th and 5th days of rooting were defined as the rooting period in which the best response to auxin was obtained. The first root formation occurred 7 days after transferring the shoots of easy-rooted varieties into the rooting media.

In the rooting experiment on Hedelfinger and Sam cherry varieties using agar solidified modified MS medium, Paul and Feucht [1985] investigated the effect of IBA, NAA and IAA on rooting. In accordance with our findings, IBA was the most effective auxin on rooting of cherry varieties. Mean rooting ratio of the two hard-rooted cherry varieties was 90%. Sam cultivar rooted at high ratio in all IBA concentrations (0.5-0.75 and 1.0 mg L^{-1}), while Hedelfinger cultivar had the highest rooting ratio with 1.0 mg L^{-1} IBA concentration. The rooting study of Snir [1982] on cherry showed that the highest rooting of *in vitro* shoots was achieved in 1/2 diluted MS medium which pH set to 5.3 containing 1 mg L-1 IBA, 2% sucrose and 0.7% agar. In compatible with our findings, Pevalek-Kozlina and Jelaska [1987] reported that the best rootings of *in vitro* and side shoot obtained from wild cherry rootstocks were achieved after immersion in a nutrient media containing 4.9 µM IBA and 2.46 IBM IBA solutions, respectively. Özzambak and Schmidt [1991] reported that *in vitro* shoots of Early Burlat and Viola cherry varieties and F 12/1 and 209/1 rootstocks were better rooted in a 1/2 strengthened media containing 1.0 mg L^{-1} IBA. The difference between K5 medium, which had the best results in our experiment and the media used by Özzambak and Schmidt [1991] was the concentration of IBA. The K5 media contained 2 mg L^{-1} IBA instead of 1.0 mg L^{-1} IBA. In another study conducted by Özzambak and Hepaksoy [1997], cherry shoots were rooted in 1/2 MS nutrient media containing 5.5-11.0-16.5 and 22.0 µM IAA, IBA and NAA. The rooting was achieved by using the specified amounts of plant growth regulators. In contrast to our findings, they reported that root color, shape, thickness and callus formation ratios were better in the presence of IAA compared to the other plant growth regulators.

In addition to the MS nutrient media of K1 containing 0.1 mg L^{-1} Thiamin in the standard content, 1.4 mg L^{-1} more Thiamin was added into the K2 media. The rooting in K2 reached 53% whereas it was only 27% in K1 media. The results revealed that addition of Thiamin to the nutrient media positively affected the rooting of the micro shoots. Kamali et al. [2006] tested a similar media to K2 in rooting studies. The rooting ratio of GF 677 and different almond rootstocks in LS media

containing 0.3 mg L^{-1} NAA and 1.6 mg L^{-1} Thiamin was 80%, which was higher than our findings (27%) obtained in 0.3 mg L^{-1} Thiamin added K1 media. Esmenjaud et al. [1993] investigated the effect of Thiamin content on rooting of Myrobolan plums in a media similar to K1. The researchers, who reached a different conclusion than our findings, reported that successful root formation was achieved in MS media containing 0.4 mg L^{-1} Thiamin and 0.5 mg L^{-1} IBA and only 0.4 mg L^{-1} Thiamin.

In this study, shoot samples were taken from the Weiroot 158 clone rootstocks used in cherry-cherry production and the shoot formation method, which is one of the tissue culture techniques was used to obtain shoot tips and then micro shoots were rooted. The *in vitro* plantlets were transferred to the vials. The vials were covered with a nylon cover and placed in the climate chamber after irrigation. Thus, only the temperature was controlled in the climate room. The experiment was continued up to the acclimation stage, the final stage of the micropropagation, and terminated.

Acknowledgement

This study was produced from the PhD thesis and funded by Ege University Scientific Research Projects Coordinator with the project number of 07-ZRF-002.

5. REFERENCES

Aka-Kaçar, Y., Yılmaz, N., Yalçın-Mendi, Y., Küden, A., Çetiner, S., 2001. *Effects of different solidifying agents and different pH levels used in in vitro media on growth of some cherry (Prunus avium L.) rootstocks.* I. Stone Seed Fruit Symposium. September 25-28, 2001, Yalova,161-166 (in Turkish).

Al-Sabbagh, M., Abdul-Kader, A., Khoder M., Kalhout A., 1999. *In vitro propagation of semi-dwarfing cherry rootstock.* An international journal on biotechnology of higher plants. Kluwer academic publishers. Plant cell, tissue and organ culture. 78(2), 173-181.

Arıcı, Ş.E., 2008. *Propagation of Some Stone Seed Fruit Rootstocks with Tissue Culture.* Süleyman Demirel University Faculty of Agriculture Journal. 3(1), 19-23. (in Turkish).

Bürün, B., Türkoğlu, G., 1996. *Meristem and Shoot Tip Cultures (1). Meristem and Shoot Tip Culture Applications* Yüzüncü Yıl University, Journal of Agricultural Faculty. 6 (3), 103-111 (in Turkish)

Büyükyılmaz, M., Öz, F., 1994. *Rootstocks used in deciduous fruit species.* Atatürk Horticultural Research Institute, Publication No: 70. (in Turkish).

Epstein, E., Zilkah, S., Faingersh, G., Rotebaum, A., 1993. *Transport and metabolism of indole-3-butyric acid in easy and difficult to root cuttings of sweet cherry (Prunus avium L.).* Acta Hort., 329, 292-295.

Espinosa, A.C., Pijut, P.M., Michler, C.H., 2006. *Adventitious shoot regeneration of Prunus serotina in vitro cultures.* Hortscience 41(1), 193-201.

Esmenjaud, D., Minot, J.C., Voisin, R., Salesses, G., Poupet, R., Onesto, J.P., 1993. *Assessment of a method using plantlets grown previously in vitro for studying resistance of Prunus cerasifera Ehr. (Myrobolan Plum) to Meloidogyne spp.* Nematropica, 23(1), 41-45.

George, E.F., Hall, M.A., De Klerk, G.J., 2008. *Plant Propagation by Tissue Culture*. 3rd Edition, Volume1. The Background, Chapter 1, p: 1-28, Springer.

Gürel, S., Gülşen, Y., 1998. *The Effects of IBA and BAP on In Vitro Shoot Production of Almond (Amygdalus communis L.).* Turkish Journal of Botany. 22, 375-379.

Hartmann, H.T., Kester, D.E., 1983. *Plant propagation principles and practices.* 3rd Ed. Printice

Hall, Inc. London, 727 p.

Kamali, K., Majidi, E., Zarghami, R., 2001. *Micropropagation of GF-677 rootstocks (Prunus amygdalus x P. persica).* Chaiers Options Mediterranean. 175-177.

Kamali, K., Majidi, E., Zarghami, R., Arvin, M.J., 2006. *Differences in micropropagation of vegetative rootstock (GF 677) and other almond seed genotypes.* ISHS Acta Hort. 726.

Karvar, S. Gülşen, Y., 1990. *The effects of almond (Prunus amygdalus Batsch.) nutrient media content on shoot yield in in vitro vegetative propagation.* Ankara University, Agricultural Faculty, Master Thesis. Ankara (in Turkish).

Murashige, T., Skoog, F., 1962. *A revised medium for rapid growth and bioassay with tobacco tissue cultures.* Physiol. Plant. 15, 473-497.

Özzambak, E., and Hepaksoy, S., 1997. *Investigations on in vitro rooting and acclimatization of sour cherry cv. Heimanns Rubinweichsel.* Acta Horticulturae. 447, 153-154.

Özzambak, E., Schmidt, H., 1991. *In vitro and in vivo micrografting of cherry (Prunus avium L.)* Gartenbauwissenschaft. 56(5), 221-223.

Pevalek-Kozlina, B., Jelaska, S., 1987. *Microclonal propagation of Prunus Avium L. symposium on in vitro problems related to mass propagation of horticultural plants.* ISHS Acta Hort. 212, 599-602.

Paul, L., Feucht, W., 1985. *Rooting sweet and sour cherry cultivar and clones in vitro.* Hort. Abst. 55(9), 679.

Pakyürek, M., Hepaksoy, S., 2019. *A Study on In Vitro Propagation Possibilities of Some Clone Rootstocks of Prunus Species.* International Journal of Scientific and Technological Research. 5(9), 65-84.

Ruzic, D., Cerovic, R., Ystaas, J., 1998. *Influence of agar brands and concentration on in vitro shoot multiplication of the cherry rootstock Gisela 5.* Acta Hort. 468, 209-216.

Ruzic, D., Saric, M., Cerovic, R., Culafic, L., 2000. *Relationship between the concentration of macro elements, their uptake and multiplication of cherry rootstock Gisela 5 in vitro.* Plant cell, tissue and organ culture. 63, 9-14.

Sauer, A., 1985. *In vitro propagation of Prunus avium L. and storage of in vitro derived plantlets.* Acta Hort. 169, 351.

Snir, I., 1982. *In vitro propagation of sweet cherry cultivars.* Hort Science, 17, 192-193.

Tang, H.R., Ren, Z.L., Renstle, G., Krczal, G., 2002. *Plant regeneration from leaves of sweet and sour cherry cultivars.* Sci. Hortic. 93, 235-44.

Zainel, A.A., Hepaksoy, S., 2018. *Investigating the Possibilities of Tissue Culture Propagation of an Idris Rootstock Pontaleb.* Ege Univ. Faculty of Agriculture Journal, 55(1), 83-88.

Zilkah, S., Faingersh, E., Rotbaum, A., 1992. *In vitro propagation of three MxM (Prunus avium x P. mahalep) cherry rootstocks*. International symposium on propagation of ornamental plants. ISHS Acta Hort. 314, 201-208.