A RESEARCH ON MICROPROPAGATION OF PIXY ROOTSTOCK

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Abstract

Clonal rootstocks have ability to produce fruits in a very short period after planting, therefore are preferred by farmers in establishing an orchard. Genetically identical origin and disease-free rootstocks can be obtained by *in vitro* techniques under laboratory conditions. The purpose of study was to investigate the reproduction possibilities of Pixy, an important plum clone rootstock used in plum and apricot cultivation, using the tissue culture techniques. The shoot tips of Pixy rootstocks were used as explants and Murashige-Skoog (MS) containing 30 g L^{-1} sucrose and 7 g L^{-1} agar was used as the nutrient medium. The shoot tips cleaned and placed on MS medium were transferred into the culture room with well controlled temperature and lighting. The samples were sub-cultured onto the same medium once in a month during shoot formation and rooting stages. The number of explants, leaves and sprouting were recorded once in a 20 days. The effects of seven different MS media were investigated during the shoot multiplication stage. Shoot multiplication was at the highest rate when a medium enriched with 4.4 µM BAP + 0.49 µM IBA + 0.29 µM GA_3 + 1mM PG. The efficiency of five different MS media were investigated during the rooting stage. The results indicated that the highest rooting performance of Pixy rootstocks can be obtained in the nutrient medium containing 1/2 MS + 2 mg/L NAA.

Keywords: Pixy (*Prunus institia* L.), plum clone rootstock, *in vitro* propagation, tissue culture, plant growth regulators.

1. Introduction

Plum production is commonly carried out with seedling rootstocks. Myrobolan rootstocks, which are the wild forms of plums, are preferred as the seedling rootstock. However, the use of clone rootstock in plum cultivation is becoming more common. The most important of these rootstocks are Pixy, Myrobolan 29-C, Myrobolan B, Myrobolan GF 31, Marianna 2624, Marianna GF 8-1, Saint Julien A, Saint Julien 655/2 and GF 677, which is a hybrid of peach and almond.

Rootstocks of stone fruits can be produced with seeds and cuttings. However, micro propagation technique, which is independent of the season, has become preferred today due to the lack of an exemplary plant in seed production and the difficulties in obtaining plants free of various diseases and viruses in cutting production. Therefore, many studies have been carried out by different researchers on the micro propagation of Prunus species [Jones and Hopgood, 1979; Ruzic and Vujovic 2008; Perez-Tornero et al. 2001; Canlı and Demir 2014; Geyik and

Canlı, 2015; Alanagh et al. 2014; Arab et al. 2014; Zainel and Hepaksoy, 2018; Pakyürek and Hepaksoy, 2019a; Pakyürek and Hepaksoy, 2019b]. Macro and micro elements and vitamins of MS nutrient medium are generally used in rootstock production using tissue culture technique [Skirvin, 1984]. Sucrose at a rate of 20-30 g L^{-1} is added to the nutrient medium as an energy source. Solid medium is preferred more than liquid medium in *Prunus* species, thus 6-8 g L^{-1} agar is used to solidify the medium. The concentrations of plant growth regulators added to the culture medium differ with respect to the culture stage and plant type. [Borkowska and Szczerba, 1991; Morini et al., 1992].

Auxin and cytokinins, which are among the plant growth regulators, are active in root and shoot bud formations from plant tissues. High ratio of cytokinin/auxin induces formation of shoots; high ratio of auxin/cytokinin induces formation of roots; the same ratio of auxin and cytokinin promotes callus formation. BAP is a commonly and successfully used cytokinin for shoot proliferation in clonal propagation, and addition of 1-2 mg L^{-1} to the medium is sufficient. The auxins used are IAA, NAA and IBA. The amount of auxins needed for the nutrient medium during the shoot reproduction period ranges between 0.1 and 1.0 mg L^{-1} [Werbrouck and Debergh, 1994]. Yancheva et al. (2003) reported that IAA, IBA and NAA have an inducing effect on shoot regeneration.

Micro propagation begins with the planting of surface sterilized shoot tip taken as an explant from the plant into the nutrient medium (Rosati et al., 1980; Sauer 1985; Bandok et al., 1989; Borkowska, 1990). After this stage, multiplication can be achieved by re-culturing of nodes containing buds, located at long shoots formed resulting from growth of shoot ends (single node culture), or by the formation of a lateral branch from the sleeping buds on the axil of leaf of the shoot tips with the application of cytokinin (Rowe, 1986; Zimmerman, 1991). The use of micro propagation techniques in clonal rootstock production in Turkey is becoming widespread in universities, research institutes located in the agriculture ministry and in the private sector laboratories. The production of clonal rootstock should be increased to prevent the losses of foreign currency by importing clone rootstocks. Therefore, this study was conducted to investigate the possibility of Pixy clone rootstock propagation by tissue culture technique under laboratory conditions.

2. Material and Methods

2.1. Material

The experiments were carried out between 2007 and 2009 in Tissue Culture Laboratory of Horticulture Department in Agricultural Faculty of Ege University. The shoot tips used as explants were collected from Pixy clone rootstocks in Fruit Research Institute located in Eğirdir town of Isparta province, Turkey. Pixy *(Prunus institia* L.*)* is a dwarf clone rootstock developed among St. Julien d'Orleans clone populations at East Malling Research Station and released in the 1970. Plum trees grafted on rootstocks starts to produce large and high-sugar fruits in the second year. Pixy is compatible well with all plum varieties and provides 30% stunting compared to St. Julien A. Approximately 1000 to 2000 trees can be planted per hectare in plum orchards where this rootstock is used [Büyükyılmaz and Öz, 1994].

2.2. Methods

Murashige and Skoog (MS) medium [Murashige and Skoog, 1962] which is widely preferred plant tissue culture growing medium was also used as nutrient medium. The MS nutrient medium was enriched by plant growth regulators belonged to three different groups. The plant growth regulators used in this study were indole butyric acid (IBA) and naphthalene acetic acid (NAA) (auxin group); gibberellic acid (GA_3) (gibberellin group) and 6-benzylaminopurine (BAP) (cytokinin group). In addition to plant regulators, nutrient medium contained 30 g L^{-1} sucrose + 7 g L^{-1} agar in the initial stage and 6 g L^{-1} agar in the rooting stage. Sodium hydroxide (1N, NaOH) and hydrochloric acid (1N, HCl) were used to adjust the pH of nutrient medium prepared using sterile purified. The nutrient medium was transferred into the sterilized glass tubes or jars, maintained in an autoclave at 121 °C temperature and 1.2 psi of pressure for 20 min and sterilized. Seven MS nutrient media were used in the multiplication stage and five MS media were used in the rooting stage of the study. The information on the nutrient medium, the amount of plant growth regulators, adjusted pH values and citations were presented in Table 1 and Table 2.

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

Medium	Contents of plant growth regulators	pH	Reference
S 1	MS + 1 mg L^{-1} BAP + 0.1 mg L^{-1} NAA	5.6	Özzambak and Hepaksoy,
S 2	MS + 1 mg L^{-1} BAP + 0.1 mg L^{-1} NAA + 0.1 mg L^{-1} GA_3	5.6	Ruzic et al., 1998
S 3	MS + 1 mg L^{-1} BAP + 0.1 mg L^{-1} IAA + 0.1 mg L^{-1} GA_3	5.6	--------
S 4	MS + 2 mg L^{-1} BAP + 0.1 mg L^{-1} IBA + 0.25 mg L^{-1} GA_3	5.6	Hepaksoy, 2004
S 5	MS + 1 mg L^{-1} BAP + 0.5 mg L^{-1} IBA + 0.25 mg L^{-1} GA_3	5.6	Hepaksoy and Tanrısever,
S 6	MS + 4.4 mM BAP + 0,49 mM IBA + 0.29 mM GA_3 + 1mM PG	5.6	Hammatt and Grant, 1997
S 7	MS + 1 mg L^{-1} BAP	6.2	Özzambak and Schmidt, 1991

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

Medium	Contents of plant growth regulators	pH	Reference
K 1	MS + 0.3 mg L^{-1} NAA	5.6	Kamali et al., 2001
K 2	MS + 0.3 mg L^{-1} NAA + 1.4 mg L^{-1} Thiamin	5.6	Kamali et al., 2001
K 3	MS + 0.5 mg L^{-1} IBA + 283.72 mg L^{-1} PG	5.7	Paul and Feucht, 1985
K 4	MS + 0.5 mg L^{-1} NAA + 283.72 mg L^{-1} PG	5.7	Paul and Feucht, 1985
K 5	1/2 MS + 2 mg L^{-1} IBA	5.6	Tang et al., 2002

2.2.1. Collection, planting and sterilization of plant samples

Shoot tips of plums were collected in the early mornings of the May. The samples were covered with wet paper towels and placed in plastic bags. The samples were transferred to the laboratory within a freezer to prevent water losses of tissues. The plant specimens were carefully cleaned to remove small leaves and washed under tap water to decrease density of microorganisms. The

pre-sterilization of samples was continued by washing to remove dirt. The samples were placed in a soapy water, occasionally stirred, waited for 20 min., and then washed under tap water for 20 min. Following the pre-sterilization, the shoot tip samples have been placed into a laminar airflow cabinet (sterilized in a horizontal and vertical plane). The samples were sterilized by soaking in 1/5 diluted solution containing 4% sodium hypochlorite for 20 min. The sterilization of shoot tip samples was accomplished by washing the samples 3 times in a sterile pure water for 5 min. to remove the disinfectant. The sterilized explants were ready for placing on the MS medium [Karvar and Gülşen, 1990].

2.2.2. *In vitro* culture conditions

During the initial, propagation and rooting stages, the explants were cultured under aseptic conditions. The explants were maintained in a culture chamber at 24±1 °C temperature, 16-h light and 8-h dark conditions. The glass tubes were used as culture containers. The mouth of tubes were closed with lids, and stretch film was used to cover the petri dishes and glass jars; therefore moisture was not controlled in the culture chamber.

2.2.3. Experimental layout and analysis of data

Experimental layout during shoot propagation and rooting stages in petri dishes and glass jars was randomized plots with three replications. Five explants were planted on each of culture plate.

The differences in mean values obtained in different nutrient media and the measurement periods and the interactions between the media and the time were assessed by variance analysis (ANOVA). Duncan's multiple range ($P<0.05$) was used as post-hoc when differences were significant in ANOVA test. The angle values of percentages were used in statistical analysis. Minitab Software was used for all statistical analysis.

3. Results

3.1. Initiation and propagation stage

Sterilized shoot tips were placed into four different MS medium. The infection, tissue blackening and drying caused to lose some of the rootstocks in the cultures. The number of losses was not high to harm the reliability of the experiment, therefore studies continued with the healthy cultures. In addition, the vitrification problems occasionally have been encountered in cultures. However, the problem did not cause a significant loss; thus, treatment was not used for the vitrification problem. Initial explanted cultures (Figure 1) were subcultured every for four weeks and transferred to fresh medium for replications. After producing sufficient number of *in vitro* shoots, the medium experiments were started. The length of growing shoots were measured and the number of leaves and number of tillerings were counted on day 0, 20, 40 and 60 of the experiment.

Figure 1. An image from *in vitro* culture studies during shoot multiplication stage of the experiment.

In vitro shoot growth in 7 MS nutrient medium containing different Pixy rootstock plant growth regulator contents was monitored during the 60-day trial. The environment x time interaction had statistically significant effect (P≤0.05) on shoot growth of rootstocks. The mean values obtained were shown in Table 3. The shoot growth of the Pixy rootstock has increased steadily for 60 days only in S 6 medium. The average shoot length values in S 7 medium increased until the day 40, though the values decreased due to the drying of the micro shoots. The average shoot length values in S 2, S 3 and S 5 medium increased until 20th day and then decreased gradually. Similarly, shoot growth in S 1 and S 4 medium increased until 20 days; however, all the shoots died on the 40th day. The highest average shoot length value for Pixy rootstock was recorded on day 20 in S 1, S 2, S 3 and S 5 medium, on day 40 in S 7 medium and on day 60 in S 6 medium. The highest mean shoot length (18.20 mm) was obtained in S 6 medium on day 60, whereas the shortest mean shoot length (1.07 mm) was recorded in S 5 medium on day 60. The average shoot length value in the S 6 environment at the beginning of the experiment was 13.13 mm and increased to 18.20 mm at the end of the trial, which corresponded to an increase of 38%. The highest average shoot length value (16.17 mm) was obtained in the same environment.The increase in average shoot length in other media was not steady due to drying and deaths of the shoots. The shoot growth in S 1 and S 4 media were weaker compared to other media.

Table 3. Average shoot length (mm) values of Pixy rootstocks in different nutrient media.

Medium	Average length of shoots (mm)			
	Day 0	Day 20	Day 40	Day 60
S 1	11,20 bcdefghıj	11,20 bcdefghıj	0,00 k	0,00 k
S 2	15,07 abcdef	16,13 abcd	16,00 abcde	5,53 jk
S 3	14,27 abcdefgh	15,07 abcdef	7,20 ıj	5,53 jk
S 4	14,20 abcdefgh	13,07 abcdefghı	0,00 k	0,00 k
S 5	12,40 abcdefghı	12,47 abcdefghı	8,07 hıj	1,07 k
S 6	13,13 abcdefghı	16,33 abc	17,00 ab	18,20 a
S 7	8,73 gıj	9,87 defghıj	10,40 cdefghıj	8,87 fghıj

Mean number of Pixy rootstock leaves in *in vitro* conditions significantly (P<0.05) differed with the environment x time interaction. The mean number of leaf values recorded were presented in Table 4.

Table 4. Average number of leaves (leaf/explant) of Pixy rootstock in different nutrient media.

Medium	Average number of leaves (leaf/explant)			
	Day 0	**Day 20**	**Day 40**	**Day 60**
S 1	4,07 fghı	4,07 fghı	0,00 ı	0,00 ı
S 2	10,40 abc	13,20 a	12,27 ab	4,80 efgh
S 3	8,87 abcdef	9,67 abcd	5,80 cdefg	4,67 efgh
S 4	7,07 cdefg	6,60 cdefg	0,00 ı	0,00 ı
S 5	7,67 cdef	8,07 bcdef	5,33 defg	0,80 hı
S 6	7,07 cdefg	8,73 abcdef	9,27 abcde	9,87 abcd
S 7	6,40 cdefg	7,47 cdef	7,40 cdefg	6,53 cdefg

Similar to the shoot length, a regular increase in the number of leaves during the trial was obtained only in the S 6 medium. The average values in S 2, S 3, S 5 and S 7 media increased until the day 20 and then a decrease was recorded. The mean number of leaves was not increased at all in S 4 medium. The values have always decreased due to deaths of the shoots and there were no living shoots on the day 40 in S 1 and S 4 media. The highest average number of leaf (13.20 leaf/explant) value was obtained on the day 20 in S 2 medium. The lowest mean value (0.80 leaf/ explant) was noted in S 5 medium. The highest mean number of leaves was obtained on day 0 in S 1 and S 4 media, on day 20 in S 2, S 3, S 5 and S 7 media and on day 60 in S 6 medium. The leaf formation in S 1 and S 4 media was considered insufficient.

The mean number of tillers statistically (P<0.05) differed with the nutrient medium. The average values obtained for the number of tillers were shown in Table 5.

Table 5. The mean number of tillers (tiller/ explant) of Pixy rootstock according to nutrient media.

Medium	Mean number of tillers (tiller/explant)			Mean
	20.gün	**40.gün**	**60.gün**	
S 1	0,00	0,00	0,00	0,00 c
S 2	0,00	0,00	0,00	0,00 c
S 3	0,00	0,00	0,07	0,33 ef
S 4	0,00	0,00	0,00	0,00 c
S 5	0,00	0,00	0,00	0,00 c
S 6	0,47	1,20	1,73	1,13 a
S 7	0,53	0,87	0,87	0,75 b

Similar to the shoot length and number of leaves in Pixy rootstock, regular increase in the number of tillers was recorded only in S 6 medium. The number of tillers in the S 7 medium increased up to the day 40 and this increase was maintained till day 60. The tiller formation in S 3 medium was not observed on day 20 and 40, while the number of tillers on the day 60 was 0.07 tiller/explant. The highest mean number of tiller was obtained on day 60 (1.73 tiller/explant) in S 6 medium. The lowest mean number of tiller was recorded on the day 60 (0.07 tiller/explant) in S 3 environment. The effects of the nutrient media tested on the shoot propagation and growth of the Pixy rootstock were different. The S 6, S 2 and S 3 media provided the best results in terms of shoot length and the number of leaves, while the S 1 and S 4 media were considered less successful. The shoot propagation for Pixy rootstock was not occurred in S 4, S 1, S 2 and S 5 media due to the lack of tiller formation. The best shoot growth occurred in S 6 and S 7 environments, respectively. The success of the media can be observed in Figure 2.

(a) **(b)**

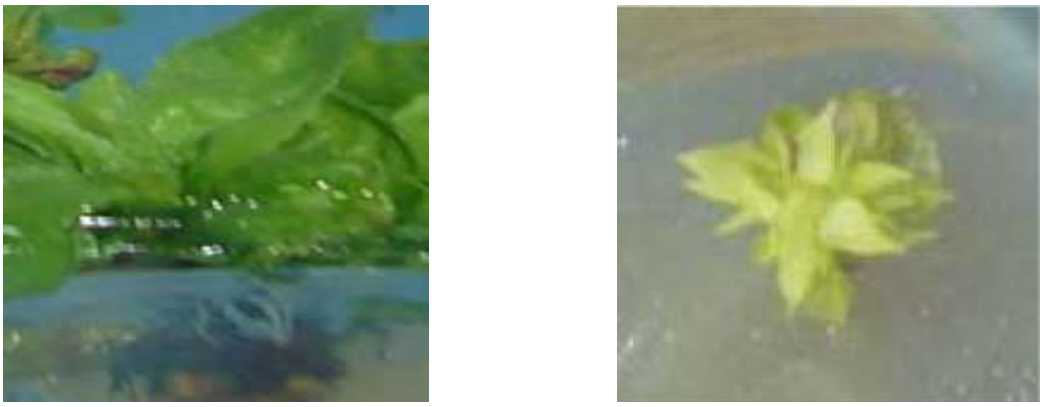

Figure 2. Growth status of Pixy rootstocks in S 6 (a) and S 4 (b) medium at the end of the 5th week.

3.2. The rooting stage of rootstocks

Five MS nutrient media containing plant growth regulators were used in rooting of Pixy clone rootstock micro shoots (Table 2). The mean rooting ratio values obtained in five media were presented in Table 6.

Table 6. Mean rooting ratios of Pixy rootstock in five different nutrient media.

Medium	Contents of plant growth regulators	pH	Ratio of Rooting (%)
S 1	MS + 0.3 mg L^{-1} NAA	5.6	33
S 2	MS + 0.3 mg L^{-1} NAA + 1.4 mg L^{-1} Thiamin	5.6	53
S 3	MS + 0.5 mg L^{-1} IBA + 283.72 mg L^{-1} PG	5.7	60
S 4	MS + 0.5 mg L^{-1} NAA + 283.72 mg L^{-1} PG	5.7	20
S 5	1/2 MS + 2 mg L^{-1} IBA	5.6	80

The first root formations of the Pixy clone micro shoots were observed on the day 5. The rooting

occurred on the days 7, 10, 12 and 15, respectively. The latest root formation occurred on the day 20. No further rooting observed after the day 20. Some of non-rooting plants dried up and other maintained growth status. The best rooting rate (80%) was obtained for this rootstock was obtained in S 5 medium and followed by S 3 (60%), S 2 (53%) and S 1 (33%) media. The lowest rooting rate (20%) in this study was recorded in S 4 medium. The image for the best rooting of a Pixy rootstock in S 5 medium (1/2 MS + 2 mg L^{-1} IBA pH=5.6) was presented in Figure 3.

Figure 3. A Pixy rootstock rooted in S 5 medium.

4. Discussion and Conclusion

In this study, explants of shoot tips were cultured in 7 MS nutrient media containing plant growth regulators with different contents to determine the ideal multiplication conditions of Pixy clone rootstocks in tissue culture. The results in the nutrient media during the shoot multiplication and rooting stages were different. The S 1, S 2, S 3, S 4, S 5, S 6 and S 7 media were tested for this rootstock during the shoot multiplication stage. The S 6, S 2 and S 3 media provided the best results in terms of shoot length and number of leaves, while the lowest mean shoot length and number of leaves was recorded in S 1 and S 4 media. The tiller formation did not occur in S 4, S 1, S 2 and S 5 media, therefore, the Pixy rootstock did not propagate in these media. The best shoot growth was recorded in S 6 and S 7 media. The Pixy rootstock reached the highest amount of proliferation in the S 6 medium, while the least multiplication was recorded in S 1 and S 4 media. The success of S 2 medium in shoot elongation and leaf formation and the fail in tiller formation indicated that S 2 medium is suitable for the initial stage and is not suitable for the multiplication stage. The S 1 medium (1 mg L^{-1} BAP + 0.1 mg L^{-1} NAA) provided very little shoot elongation in Pixy rootstock. Sauer (1985) conducted a study on the Mazzard cherry rootstock and reported satisfactory results about the S 1 medium. The researcher reported the development of side shoots when meristems of the apical buds on young shoots were cultured in MS nutrient medium containing 2 mg L^{-1} BAP and 0.1 mg L^{-1} NAA. Similar results were obtained in an in vitro shoot multiplication study conducted by Özzambak and Hepaksoy (1997) in Heimanns Rubinweichsel sour cherry variety. The Highest shoot multiplication was recorded in MS nutrient medium containing 0.5 mg L^{-1} BAP and 0.1 mg L^{-1} NAA. In this study, the MS medium containing 1.0 to 1.5 mg L^{-1} BAP was considered

the ideal subculture medium. Similar results were obtained when the same amount of IAA, IBA and NAA were applied instead of BAP.

The S 2 (1 mg L^{-1} BAP + 0.1mg L^{-1} NAA + 0.1 mg L^{-1} GA_3) and S 3 (1 mg L^{-1} BAP + 0.1 mg L^{-1} IAA + 0.1 mg L^{-1} GA_3) media and all groups containing cytokinin (BAP), auxin (NAA and IAA) and gibberellin (GA_3) groups are plant growth regulators. In our study, the S 3 medium containing IAA differently from S 2 environment was tested to investigate the effects different auxin uses on multiplication. The results revealed that the S 2 and S 3 media considered among the best shoot multiplication media in Pixy rootstock. Pixy rootstock provided better results in the presence of NAA, ie S 2 medium. Similarly, Ruzic et al. (1998) emphasized that cherry rootstocks provided the best multiplication in MS environment, which had the same plant growth regulator content as in S 2 medium. In another experiment conducted by Ruzic et al (2000) in Gisela 5 rootstocks, MS macro elements were increased at 2, 1/2 and 1/4 fold strengths respectively, 3.4 µM BA + 0.5 µM NAA + 0.3 µM GA_3 was added and the pH was adjusted to 5.78. The best shoot multiplication and growth occurred in the medium where macro elements were increased at 2 fold strength. In parallel with our study, Arıcı (2008) investigated the multiplication possibilities of Maxma-14 and GN rootstocks used for stone fruit varieties with shoot tip and side shoots. The researcher reported successful shoot multiplication results with the nutrient medium containing 2.0 mg L^{-1} BAP + 0.2 mg L^{-1} NAA + 0.5 mg L^{-1} GA_3 for Maxma-14 rootstock, and 1.0 mg L^{-1} BAP + 0.02 mg L^{-1} NAA + 0.5 mg L^{-1} GA_3 for GN rootstock. The S 4 (2 mg L^{-1} BAP + 0.1 mg L^{-1} IBA + 0.25 mg L^{-1} GA_3) and S 5 (1 mg L^{-1} BAP + 0.5 mg L^{-1} IBA + 0.25 mg L^{-1} GA_3) media also contain plant growth regulators from the three main groups. Unlike S 2 and S 3 media, S5 and S 5 contain IBA. The results revealed that Pixy rootstock did not provide good multiplication in S 4 media and became prone to vitrification. High level of BAP (2 mg L^{-1}) in S 4 medium had a negative impact on shoot growth of these rootstocks. In parallel with this finding, Gürel and Gülşen (1998) reported that high BAP (2 or 3 mg L^{-1}) concentration led to decreased vitrification and viability in shoots. Hepaksoy and Tanrısever (2004) reported successful shoot multiplication for Gisela 5 and Gisela 6 rootstocks in the MS media which was similar to the S 5 medium.

The effect of PG on shoot multiplication was investigated in S 6 environment. S 6 (4.4 µM BAP + 0.49 µM IBA + 0.29 µM GA_3 + 1 mM PG) medium contains PG (Floroglycinol). The Pixy rootstock provides good shoot growth in this medium. In addition, the growth of Pixy rootstock in S 5 medium (4.4 µM BAP + 0.49 µM IBA + 0.29 µM GA_3), which had the same plant growth regulatory content as the S 6 medium was also good. Phenolic substances secreted by the explant tissue into the nutrient medium cause blackening in the medium and prevent the growth of explant. Pontikis and Melas (1986) recommends adding fluroglycinol to the nutrient medium to reduce or eliminate this effect of phenolic substances. In a study supporting the PG application, Hammatt and Grant (1993) conducted a study with the shoot tip method in F 12/1 (*Prunus avium*) and Colt (*P. avium x P. pseudocerasus*) cherry rootstocks. The shoot multiplication was increased when the nutrient medium contained 2.2 µM BA and 1.0 µM fluoroglycinol (PG), 5.5 or 6.5 g L^{-1} agar added, and pH adjusted to 5.0.

The shoot multiplication of Pixy rootstock increased in S 7 (1 mg L^{-1} BAP pH 6.2) medium. A study supporting the S 7 medium was conducted on Damil, Edabriz, Gisela 5 and MaxMa cherry rootstocks. The effects of various medium solidifying additives, and different pH levels on shoot multiplication, shoot length and weight were examined. The optimum pH level was determined as 6.2 and the best result to solidify was reported as the agar (Aka-Kaçar et al., 2001).

The statistical assessment in the shoot multiplication stage indicated that the number of tillers for the rootstocks tested in nutrient media is low. The number of tillers was low in media where no living shoots remained after 60 days due to the drying of some micro shoot tips during the experiment and the death of some shoots completely. Although different media have come to the fore based on the statistical evaluations, the observations made during the trial revealed that the best shoot multiplication was generally in S 5 medium containing 1 mg L^{-1} BAP + 0.5 mg L^{-1} IBA + 0.25 mg L^{-1} GA_3 plant growth regulator.

Different results were also obtained from the nutrient media at the rooting stage of the study. The most successful medium was the S 5 containing 2 mg L^{-1} IBA as auxin and MS applied at 1/2 strength. Similarly, Al-Sabbagh et al. (1999) confirmed that successful rooting occurs in media containing IBA or NAA. The researchers reported that rooting occurred after 4 weeks with the addition of 0.49 µM NAA or 0.49 - 2.45 µM IBA in solid and liquid MS media. Epstein et al. (1993) investigated the transport and metabolism of IBA in aseptic conditions. The days 2, 3, 4 and 5 were defined as the rooting period for cherry varieties with different rooting characteristics, and the first root formation was observed 7 days after transferring the shoots of the easily rooted varieties into the rooting medium.

In the rooting experiment on Hedelfinger and Sam cherry varieties conducted by Paul and Feucht (1985), agar-solidified modified MS medium was used and the effect of NAA, IAA and IBA on rooting was investigated. Similar to our results, IBA was reported as the most effective auxin on rooting. An average of 90% rooting was obtained on these two cherry varieties that are known having difficulties in rooting. The Sam cultivar had the highest rooting ratios n all IBA concentrations (0.5 - 0.75 and 1.0 mg L^{-1}), the highest rooting ratio for Hedelfinger cultivar was obtained with 1.0 mg L^{-1} IBA. Snir (1982) found that the highest rooting of vitro shoots in cherry was in MS medium (pH 5.3) diluted in the ratio of 1/2 containing 1 mg L^{-1} IBA, 2% sucrose and 0.7% agar. Pevalek- Kozlina and Jelaska (1987) stated that the best rooting was obtained after immersion of in vitro shoots into the nutrient medium containing 4.9 µM IBA and the side shoots in 2.46 µM IBA solution. Özzambak and Schmidt (1991) reported that the in vitro shoots of the F 12/1 and 209/1 of Early Burlat and Viola cherry rootstocks were well rooted in a 1/2 strength MS medium containing 1.0 mg L^{-1} IBA. The S 5 medium, which provided the best results in our study, contains 2 mg L^{-1} IBA instead of 1.0 mg L^{-1} IBA.

The rooting rate in S 2 medium containing 1.4 mg L^{-1} Thiamin different from the S 1 medium, has reached 53% and rooting of Pixy rootstocks were better than S 1 medium. The rooting rate in S 1 medium was 33%. Addition of Thiamin to the nutrient medium favors the rooting of micro shoots. Kamali et al. (2006), who tested similar medium content to the S 2 medium, reported that the rooting of GF 677 and different almond rootstocks reached to 80% in LS medium containing 0.3 mg L^{-1} NAA and 1.6 mg L^{-1} Thiamin. The effect of Thiamin content on

rooting of Myrobolan plums in a medium similar to S 1 medium was investigated by Esmenjaud et al. (1993). The findings were contradicting to our results, they presented sufficient root formation in MS media containing 0.4 mg L^{-1} Thiamin and 0.5 mg L^{-1} IBA and in a MS medium containing only 0.4 mg L^{-1} Thiamin. The highest rooting rate (80%) for Pixy rootstock in our experiment was obtained with S 5 medium and the lowest rooting rate was 20% in S 4 environment.

In our study, the shoot tips obtained from the Pixy clone rootstock used in plum production were used initially to form a shoot culture using the tissue culture technique and then the micro shoots obtained were rooted. The *in vitro* plantlets produced were transferred to the viols. The viols were covered with a plastic cover after irrigation and inserted into the climate chamber. The temperature was controlled in the climate chamber. The experiment was continued to the external stage, which is the last stage of micro propagation, and ended.

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