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Research Article

DIFFERENT APPROACHES TO *IN VITRO* ROOTING POSSIBILITIES IN *PRUNUS* ROOTSTOCKS

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ABSTRACT. The possibility of vegetative propagation is one of the most essential criteria in the breeding rootstocks. In this mode of propagation, in vitro propagation has taken the place of propagation by cuttings with the development of technology. In vitro propagation practices are used effectively by nursery enterprises. Firms try different modifications and application forms to achieve maximum success in this form of propagation. In this way, it is tried to create optimum propagation protocols. This study aimed to increase the rooting possibilities of clone rootstocks by applying different IBA dip applications. The study was carried out in the laboratories and greenhouses of the Eastern Mediterranean Transition Zone Agricultural Research Institute in 2022. At the end of the study, it was determined that the average root number per explant varies between 0-2.36 explants, and the average root length varies between 0-9.40 cm in the applied clone rootstocks. When the study results were compared with similar studies, it was determined that the results were promising. According to the results obtained from the study, it was thought that immersion in an IBA solution before rooting could be a solution for some clone rootstocks with rooting problems.

Keywords: *in vitro*, *prunus*, rootstock

INTRODUCTION

Vegetative propagation *in vitro*, in other words, micropropagation, is the most effective biotechnological method used, especially in rootstock breeding studies. Today, with the development of this method, many nursery companies are getting very successful results in *in vitro* propagation. It is reluctant the produce rootstocks that cannot achieve successful results in *in vitro* propagation, and the commercial value of these rootstocks remains low (Petri and Burgos, 2005).

Vegetative propagation possibilities of rootstocks obtained after many years of breeding work may differ. This may be due to internal factors such as hormone and enzyme activities, depending on the genetic structure of that rootstock. Although positive results were obtained in the proliferation stage of some *prunus* rootstocks, it was revealed in the studies that they formed resistance during the rooting period. For this reason, different successful methods have been developed in micropropagation studies of different prunus rootstock species. However, research has shown that some *prunus* rootstocks have more limited vegetative propagation possibilities (Espinoza et al.2006; Liu and Pijut 2008; Novak et al.2004)

In vitro propagation in deciduous plants gives successful results, especially in samples taken from nodal explants. From *Prunus* rootstocks, *P.canescens* (Antonelli and Druart 1990), *P. padus* (Hammatt 1993), *P. domestica* (Bassi and Cossio 1991; Nowak et al. 2004),

P. persica (Declerck and Korban 1996; Gentile et al. 2002)), *P. dulcis* (Ainsley et al. 2000; Miguel et al. 1996; Tang et al. 2002), *P. armeniaca* (Petri et al. 2008; Perez-Tornero et al. 2000), *P. avium* (Bhagwat and Lane 2004; Matt and Jehle 2005), and *P. serotina* (Liu and Pijut 2008; Espinosa et al. 2006; Hammatt and Grant 1998) strains. In addition, it is understood from the literature that there are successful results in micropropagation studies from leaf tissue samples other than the nodal explant (Petri and Burgos 2005; Petri et al. 2008; Ramesh et al. 2006; Song and Sink 2006). Some peach x almond hybrid rootstocks have been reported to cause rooting problems in vitro propagation. It has been reported that these problems can be an important obstacle to the commercial value of rootstocks. Although the rootstock is superior in many features, the low possibility of in vitro propagation has led researchers to try different micropropagation methods. Modification of the nutrient media or the use of different hormone combinations have been the most used methods in solving rooting problems of explants. These studies are very important in terms of establishing appropriate production protocols. However, there are cases where these protocols are not sufficient in vitro rooting studies. In such cases, it is thought that different applications may be useful. As it is known, the method of applying liquid IBA solution to the steel bases gives very successful results in propagation studies with cutting. This method is a very effective and economical method that is still used in propagation with cutting. It is thought that a similar application *in vitro* rooting stage of micro shoots have a positive effect on rooting success. The use of this method can give positive results, especially in rootstocks with low rooting success but considered superior in terms of rootstock characteristics. If such applications give positive results and the results can be applied in practice, the commercial value of superior rootstocks that cannot be propagated vegetatively will increase. This study was carried out in the Eastern Mediterranean Transition Zone Agricultural Research Institute Laboratories of the in 2022 for this purpose.

MATERIAL AND METHODS

Plant Material, Preparation of Explant and Sterilization

The plant material of the study consisted of 10 rootstocks and GN-22 control rootstock which were grown in the controlled greenhouses of the Eastern Mediterranean Transition Zone Agricultural Research Institute, and obtained by crossbreeding. 0.5-1 cm long nodal explants from these rootstocks were used *in vitro* studies (Table 1).

The explant sources of the study are the branches of the 2-3-year-old hybrid *prunus* rootstock seedling grown fully controlled greenhouse in the Eastern Mediterranean Transition Zone Agricultural Research Institute. Branches, 10-15 cm tall and have nodal segments, taken from these seedlings were brought to the laboratory after their leaves were cut. Physically concentrated dirt and microorganisms on the bark were tried to be removed by shaking with liquid soap in a container filled with water for 30 minutes in the laboratory.

Subsequently, the explants taken into the sterile cabinet were kept in 70 % alcohol for 30 seconds and washed three times with sterile distilled water to prevent the toxic effect of ethanol. In the next step, when the application of ethanol was completed, the explants were rinsed with sterile pure three times after waiting for 20 minutes in a 30% (2.5% sodium hypochlorite) commercial hypo solution containing a few drops of Tween-80 (Tween 80,

Sigma-Aldrich, USA). These washed explants were sterilized in an autoclave at 121 C for 20 minutes and then transferred to the petri dishes with sterile filter paper, and their buds were cut. Irregular and unnecessary parts of damaged tissues in aseptic conditions were removed from these sterilized explants. Later, the upper parts of explants that contained one bud was cut with a number 3 scalpel in a way that the explants were 1,5-2 cm long was implanted in the medium at a 45° angle.

Table 1. *List of promising rootstock of FG series obtained by crossbreeding*

Clone No	Origin of Rootstock	
GN-22	Garfi Almond X Nemared Peach	*(P. amygdalus X P. persica)*
FG-24 FG-36 FG-48 FG-50 FG-57 FG-58 FG-66 FG-69 FG-70 FG-71	Feragnez Almond X GN-22	*P. amygdalus X* (*P. amygdalus X P. persica)*

Shoot Proliferation and Multiplication

The explants were transferred to MS medium containing four different specific PGRs (6-Benzylaminopurine (BAP), Indol butyric acid (IBA), Gibberellin (GA_3),) for the propagation of multiple shoots after the surface sterilization was completed. Explants were cultured in a medium supplemented with BAP (0.5, 0.75, 1.0, 1.25, 1.75, 2.0 mg L^{-1}), IBA (0.1 mg L^{-1}), and GA_3 (0.2 mg L^{-1}). All the in vitro cultures were kept in the medium for 60 days in media containing these different specific PGRs for shoot proliferation and multiplication.

IBA Application to Micro Shoots After Proliferation

Micro shoots formed after proliferation were taken from the nutrient medium in a sterile cabinet and divided into 2-3 cm micro cuttings in a sterile container. 1000 ml.L^{-1} IBA solution was prepared for application. For this, 1 g of powder IBA was dissolved in 98% ethyl alcohol, and the final volume was completed to 1000 ml with distilled water. Then, 1000 mL.$^{-1}$ IBA solution, sufficient for the experiment, was sterilized using a syringe tip filter with a pore size of 0.22 µm. 10 ml syringe was used for sterilization, and the amount of liquid passing through the filter was slow in sterilization of the IBA solution. The syringe filter at the tip was changed after every 10 ml use to ensure more reliable sterilization of the solution. The syringe tip filter, glass bottle, instruments, and equipment used during sterilization were sterilized in an autoclave at 121°C for 20 minutes. The separated microcuttings were immersed in indole-3 butyric acid (IBA) for 10 seconds in groups according to the genotype. In addition, the cuttings not immersed in the IBA solution were evaluated as a control in the experiment. The immersed micro cuttings were transferred directly to the new plant growth regulator-free MS medium. Cuttings planted in the nutrient medium were incubated in a climate chamber at ± 24 °C in a 16/8 hour light/dark photoperiod. After about 1.5-2 months, the results of the rooting application were evaluated. Rooting rate (%), root number (piece), and root length (cm) values were determined as a result of the rooting experiment.

Rooting and Acclimatization

2-3 cm long nodal explants, which were obtained by emerging the control media explants, were used for the rooting experiment. Healthy explants were transferred to supplemented MS medium containing IBA (0.5, 0.75, 1.0, 1.25, 1.75, 2.0 mg L^{-1}) from the auxin group, and BAP (0.1 mg L^{-1}) from the cytokine group for root development (Table 1). White and tiny rootlets formed within two weeks, and elongation and browning in color began in the medium at the end of the 4th week in these explants taken into the rooting medium. At this stage, well-rooted plantlets (2-5 roots of length 5-12 cm), whose observations and measurements were completed, were carefully washed with water to remove agar. Then they were transplanted to viols containing sterile peat, covered with transparent plastic lids, and placed in the greenhouse. Humidity was continuously checked and maintained between % 80-85, and the temperature was set at 21-22 °C for 10-12 days. The plantlets accustomed to the environment were transferred to larger plastic pots filled with soil, sand, and peat (2: 1: 1) under normal conditions in a semi-shaded area.

Statistical Analysis

The experiment was set up in a randomized plot design with three replications. 5 jars were found in each replication plot, and three explants were found in each jar. Analysis of variance was analyzed using the JMP 5.0 package program at a 5% significance level, and multiple comparisons were analyzed with the LSD test.

RESULTS

Table 2. *Effects of IBA immersion number of root*

Rootstock	Treatment	Number of Root (Pcs/Explant)		Treatment	
GN-22 (Control)	A	0.00 ±0.00 e	0.33 ±1.25 C	A	1.56 ±2.67 A
	B	0.67 ±1.70 de			
FG-24	A	3.12 ±2.85 a	1.73 ±2.57 AB		
	B	0.35 ±1.12 de			
FG-36	A	2.27 ±2.75 abc	1.45 ±2.38 B		
	B	0.62 ±1.53 de			
FG-48	A	2.50 ±3.13 ab	1.62 ±2.74 AB		
	B	0.75 ±1.91 de			
FG-50	A	0.00 ±0.00 e	0.00 ±0.00 C		
	B	0.00 ±0.00 e			
FG-57	A	0.00 ±0.00 e	0.29 ±1.06 C		
	B	0.57 ±1.44 de			
FG-58	A	0.00 ±0.00 e	0.20 ±0.91 C	B	0.63 ±1.86 B
	B	0.40 ±1.26 de			
FG-66	A	3.32 ±3.25 a	2.36 ±3.75 A		
	B	1.40 ±3.96 bcd			
FG-69	A	3.05 ±3.53 a	2.09 ±2.98 AB		
	B	1.14 ±1.87 cde			
FG-70	A	0.50 ±0.00 de	0.25 ±1.13 C		
	B	0.00 ±0.00 e			
FG-71	A	2.91 ±2.67 a	1.70 ±2.41 AB		
	B	0.49 ±1.26 de			
$LCD_{0.05}$		**1.25****	**0.88****		**0.37****

A: IBA immersion applied. B: IBA immersion not applied.

The average root number of the selected rootstocks varied between 0-2.36 units/explant, and statistical differences were found between them at a high rate (1%) after the application.

However, all rootstocks are generally divided into two main groups. The highest root number was in FG-66 rootstock, followed by FG-69, FG-24, FG-71, and FG-48 rootstocks in the same statistical group with 2.09, 1.73, 1.70, and 1.62 units/explant values, respectively. FG-50 rootstock did not form rooting, while FG-58 rootstock rooted in 0.20 units/explant number. On the other hand, in the applications, a statistically significant difference occurred in the explants applied IBA (1.56 units/explant). It is seen that there are statistically significant differences when the interaction between the application and rootstock is examined. It was noted that there were very significant rooting increases in the clone rootstocks FG-24 (3.12 units/explant) and FG-71 (2.91 units/explant). At the same time, the application differences did not cause a significant difference in the FG-66 rootstock. However, it was observed that the application did not significantly affect the number of roots in FG-58, FG-50, and FG70 rootstocks and control rootstocks.

Table 3. *Effects of IBA immersion diameter of root*

Rootstock	Treatment	Root Diameter (mm)		Treatment	
GN-22 (Control)	A	0.00 ±0.00 d	0.10 ±0.38 B	A	0.63 ±1.09
	B	0.20 ±0.51 cd			
FG-24	A	1.16 ±1.10 a	0.66 ±0.99 A		
	B	0.17 ±0.53 cd			
FG-36	A	0.18 ±1.35 d	0.64 ±1.11 A		
	B	1.10 ±0.46 a			
FG-48	A	0.96 ±1.18 ab	0.64 ±1.01 A		
	B	0.31 ±0.66 cd			
FG-50	A	0.00 ±0.00 d	0.00 ±0.00 B		
	B	0.00 ±0.00 d			
FG-57	A	0.00 ±0.00 d	0.14 ±0.54 B		
	B	0.29 ±8.90 cd			
FG-58	A	0.00 ±0.00 d	0.09 ±0.42 B	B	0.23 ±0.59
	B	0.18 ±5.23 cd			
FG-66	A	1.38 ±14.67 a	0.81 ±1.14 A		
	B	0.23 ±3.35 cd			
FG-69	A	1.12 ±12.89 a	0.82 ±1.10 A		
	B	0.52 ±4.73 bc			
FG-70	A	0.00 ±0.00 d	0.11 ±0.52 B		
	B	0.23 ±3.37 cd			
FG-71	A	1.28 ±12.94 a	0.71 ±1.13 A		
	B	0.21 ±5.00 cd			
$LCD_{0.05}$		**0.47****	**0.33****	**0.13****	

A: IBA immersion applied. B: IBA immersion not applied.

The mean root diameters of selected and control rootstocks after IBA applications showed significant differences (1%). It was seen that the highest root diameters were in the same statistical group in FG-69 (0.82 mm), FG-66 (0.81 mm), FG-71 (0.75 mm) rootstocks in the study. It was understood that the lowest root diameter was in the FG-50 rootstock. In the applications, while the average root diameter was 0.63 mm in IBA-applied rootstocks, it was 0.23 mm in the other group. There were statistically significant differences in rootstock application interaction. While the FG-69 rootstock was not affected by the application, the FG-24 and FG-71 clone rootstocks were positively affected by the application and there was a remarkable increase in root diameters.

Table 4. *Effects of IBA immersion length of root.*

Rootstock	Treatment	Root Length (mm)		Treatment	
GN-22 (Control)	A	0.00 ± 0.00 b	0.93 ± 6.73 B	A	7.51 ±12.35
	B	1.86 ± 3.18 b			
FG-24	A	16.21 ±12.61 a	9.20 ±11.43 A		
	B	2.20 ± 5.71 b			
FG-36	A	12.73 ±14.83 a	7.80 ±11.82 A		
	B	2.86 ± 3.64 b			
FG-48	A	12.82 ±14.94 a	8.55 ±12.07 A		
	B	4.28 ± 5.71 b			
FG-50	A	0.00 ± 0.00 b	0.00 ± 0.00 B		
	B	0.00 ± 0.00 b			
FG-57	A	0.00 ± 0.00 b	1.90 ± 6.73 B		
	B	3.80 ± 8.90 b			
FG-58	A	0.00 ± 0.00 b	1.67 ± 4.05 B	B	2.87 ±5.09
	B	3.35 ± 5.23 b			
FG-66	A	16.51 ±14.67 a	9.40 ±12.87 A		
	B	2.29 ± 3.35 b			
FG-69	A	12.20 ±12.89 a	8.09 ±10.43 A		
	B	3.98 ± 4.73 b			
FG-70	A	0.00 ± 0.00 b	0.98 ± 2.61 B		
	B	1.97 ± 3.37 b			
FG-71	A	14.09 ±12.94 a	8.37 ±11.30 A		
	B	2.65 ± 5.00 b			
$LCD_{0.05}$		**5.54****	**3.92****	**1.49****	

A: IBA immersion applied. B: IBA immersion not applied.

IBA application had significant effects on average root length. The highest root length was found in the FG-66 rootstock (9.28 cm) in the study. It was observed that FG-48, FG-69, and FG-71 rootstocks were 8.58 cm, 8.53 cm, and 8.48 cm, respectively, in the same statistical group. The lowest root length was again in the FG-50 rootstock. IBA application affected the root length positively. It is seen that 7.51 cm in the application and 2.87 cm in the non-application. There were significant differences in rootstock application interactions. The highest root length was found in the A/FG-66 interaction (16.51 cm). The lowest mean root length was in the B/FG-50 interaction. It is seen that the average root length is 12 cm and above in the rootstocks in the first group, which consists of two main groups.

DISCUSSION

Zhou et al. found the number of roots per explant in different IBA doses to be 2.3-5.3 units/explant on the effect of different IBA doses on micropropagation possibilities in 2010 Nemaguard rootstock in their study. The same researchers determined the average root length as 1.5-5.4 cm, although it varies according to the IBA doses. Fotopoulos and Sotiropoulos conducted a study on the effects on rooting performance by applying different mineral concentrations with dark light on standard rootstock coded PR-204/84 of similar origin to the rootstocks used in our study. Researchers have obtained values between 0-15.2 in different mineral concentrations and light-dark studies on the average number of roots per/explant in this rootstock in their studies. In the same study, the researchers determined the average root length as 0-14.5 cm. Dejampour et al. 2011 investigated the *in vitro* propagation possibilities of HS314 coded standard rootstock and found the average number of roots per explant to be

2.5-5.5 per explant. The same study determined the average root length between 2.5 and 7.5 cm. Antonopoulo et al. 2005 researched in vitro propagation possibilities of GF-677 rootstock of different Riboflavin applications. In their studies, researchers have determined the average number of roots per explant as 0-5.6 units/explant in standard GF-677 rootstocks. In the same survey, mean root length values were found to vary between 0-5.19 cm, although it varies according to the application. Garoosi et al. 2010 found the average number of roots per explant as 0.33-2.62 units/explant on the effects of different plant growth regulators on the *in vitro* propagation of GF-677 rootstock in their study. They determined that the average root lengths varied between 0-8 cm in their same study. When these rootstock studies at various times and with similar origins are examined in general, it should be noted that our study's results show similarities. The results of the standard rootstocks used in this study are similar to the results obtained from our study.

There are difficulties in the propagation of some rootstocks in micropropagation studies. Mass propagation possibilities are sought in these rootstocks at the desired density. To do so, modifications are usually made to the components of the nutrient medium or combinations of hormones. Such practices often yield positive results. Many companies engaged in commercial propagation also create suitable protocols by using these methods. We have sought to increase the possibilities for rooting, particularly in rootstocks with rooting problems, with a different approach, unlike all these applications in this study. Immersion in IBA liquid solutions was determined to be effective for rooting before explants were removed from rooting media at the end of the study. However, it was concluded that this application did not positively affect all clone rootstocks in the same study. It is thought that positive results can be achieved with such applications in rootstocks with rooting issues in micropropagation.

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