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Investigation of propagation with hardwood cutting in different *Prunus* species

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Abstract

This study was carried out in the greenhouses of the Eastern Mediterranean Transitional Zone Agricultural Research Institute in 2019 to investigate rooting situation of clonal rootstock candidates belonging to different species of *Prunus* obtained by selection breeding from Kahramanmaraş natural plantation. In the study, 28 rootstocks were usedfrom *P.cerasifera*, *P.domestica*, *P.divaricata* and *P.persica*. GF 677 (*P. persica* × *P. amygdalus*) Myrobolan 29C (*P. cerasifera*) and Pixy (*P. institia*) were used as control rootstocks. In the experiment, 0- 1000- 2000- 3000 mg L^{-1} IBA (Indolbutyric acid) and medium size perlite were used. As a result of the study, the highest rooting percentage was obtained from *P. persica* with 98.07% and the lowest from *P. divaricata* with 74.19%. The highest number of roots was determined in *P. persica* (9.14 pieces/cutting) and the lowest number of roots was found in *P. divaricata* (3.19 pieces/cutting). Among all clones used in the study, 100.00% rooting was detected in six candidate clonal rootstocks (KL-20, KL-12, KL-21, KL-24, KL-27, KL-23). The highest mean root number was found in clone KL-27 (*P. persica*) with 14.91, and the highest root length was found in KL-59 (*P. cerasifera*) with 64.82 mm. At the end of the study, although rooting results are better in rootstocks of *P. persica* species, despite lower results in rootstocks of *P. divaricata* species, it was found interesting that the best clonal rooting result was in the clone of *P. domestica* which 1000 mg. L^{-1} and 2000 mg.L^{-1} dose IBA applications gave the best results according to both rooting rates and average root length values.

Keywords: Rootstock, steel, plum, rooting, *Prunus*

Introduction

Plum and peach are two important stone fruit species belonging to Rosacea family of *Prunus* genus, which has a growing environment in temperate and subtropic regions, one of which is homeland, Anatolia. In our country, there is a type of peach belonging to *P. persica* and there are wild plum types belonging to *P. cerasifer to, P. divaricata, P. domestica, P. spinosa, P. institia* species in the natural plantation (Ercisli, 2004). These wild genotypes belonging to both species found themselves habitat in extreme climatic conditions in the natural environment, they survived healthy individuals and were able to continue their lineage. These features are important in the use of suitable rootstock, which is one of the important parts of modern fruit growing (Zhebentyayeva et al., 2019). In fruit trees, rootstocks have been used for more than 2000 years, as it is understood from ancient Greek Hellenistic manuscripts. Clonal rootstock use started to take place in fruit trees cultivation after the 17th century. It is not possible to produce homogenous plants from the majority of temperate climate fruits because of their long heterozygous and generation periods (Jones et al., 1985; Webster et al., 1985; Guney et al., 2016; Uğur and Kargı, 2019).

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The production of fruit trees developed with different breeding techniques and with the required criteria can only be possible by grafting, cutting, etc. by vegetative propagation means. In modern fruit growing, fruit trees are usually made by grafting on generative or clonal rootstocks. The use of clone rootstocks due to the absence of homogeneus plants in grafted scions on the generative rootstocks, differences and delays in fruit set formation, differences in phytochemical developments and rootstock-scion biochemical interaction (Oldoni et al ., 2019: Guney, 2019) that its widespreadness caused rooting feature to be an important criterion in rootstock breeding studies (Sergiu et al., 2009). In many countries, peach and plum rootstocks are widely used because they are easily propagated vegetatively for rootstock fruit trees (Lepsis and Dekana 2008; Szecko et al., 2001). Although our country has a rich genetic resource in wild plum population, rootstock breeding studies started after Europe and America. Due to the richness of our genetic resources, rootstock breeding studies conducted recently have been in the form of selection breeding (Uğur and Kargı, 2019). In these studies, hardwood cuttings taken from the selected genotypes are taken to rooting in suitable rooting environments. By this way, researchers could have an idea about the possibility of vegetative propagation of the genotypes. In many studies, it has been determined that auxin hormone is effective especially in the formation of lateral roots in steel production (Gundesli, 2018). Taiz and Zeiger (2008) also reported that auxin and some internal factors positively affect the formation of adventitious roots in cutting by promoting cell division and the beneficial effects of plants in cutting production. This interaction of genetic structure and hormones differs even in individuals with the same type (Kacal and Koyuncu, 2008).

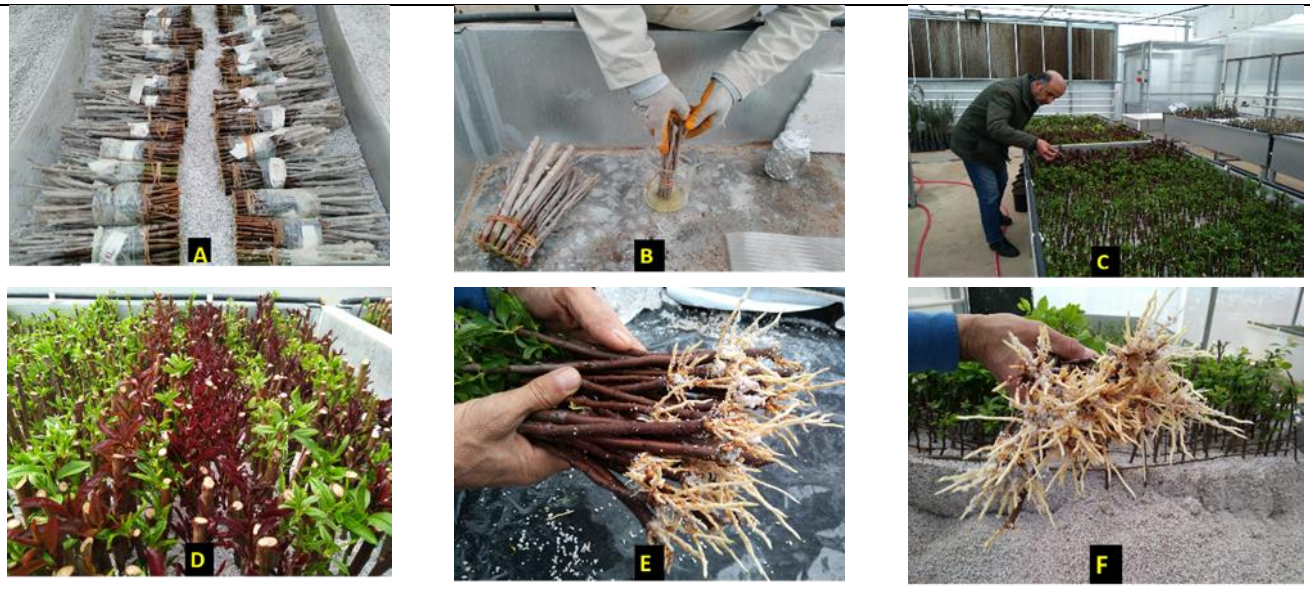
The aim of this study was to investigate the possibilities of rooting with different genotypes of different *Prunus* species and to ensure the advanced use of rootstock candidates with good rooting characteristics by comparing rooting levels with standard rootstocks.

Material and Method

The material of the study consisted of 28 selected rootstocks of different *Prunus* species obtained by selection breeding (Uğur, 2017) and control rootstocks as GF-677 (*P. persica* × *P. amygdalus*), Pixy (*P. institia*) and Myrobolan 29C (*P.cerasifera*) (Table 1). The selected control rootstocks were taken to the reproduction trial with wood cuttings, which is the main study for propagation. In November-2018, about 20-25 cm long, 6 - 12 mm thick cuttings were taken from 1-year old shoots in the dormant period of the trees and were taken to rooting as of February-2019 (Figure 1). The cuttings were kept in water with a fungicide solution with 80% Fosety- Al effective agent for a day without taking root at first. Rooting pool area of $30m^2$ ($3 m^2 \times 10$), medium size perlite with a thickness of 10 m^3 3-4 mm was used for the cuttings. The pools where the cuttings were taken to rooting environment have been applied mist application for 5 seconds per hour totally 120 seconds for whole day. Hardwood cuttings were left for rooting by applying hormone in rooting pools during 60 days with an IBA dose of 0-1000-2000-3000 $mg.L^{-1}$. At the end of this period, rooting success has been achieved. In rooting studies with hardwood cuttings, standard parameters such as rooting percentage, average number of roots and average root length were measured. Rooted cuttings, whose measurements were completed at the beginning of April 2019, were taken into pots and the development status of the steels taken into the pots was observed. All cultural processes were carried out without interruption.

Table 1. Number of clones and clone names of clone rootstock candidates by species

Species Name	Number of clones	Clone names
Prunus persica	7	KL-27, KL-25, KL-24, KL-23, KL-21, KL-20, KL-43
Prunus cerasifera	7	KL-15, KL-16, KL-17, KL-58, KL-59, KL-60, KL-61
Prunus divaricata	7	KL-4, KL-5, KL-8, KL-52, KL-10, KL-11, KL-35
Prunus domestica	7	KL-1, KL-2, KL-3, KL-6, KL-9, KL-12, KL-63
Control	3	GF-677 (*P.persica* × *P. amygdalus*), Myrobolan 29C (*P. cerasifera*), Pixy (*P. institia*)



A: Preparing the cuttings for rooting environment, B: IBA hormone application, C: Regular control of cuttings taken into rooting environment, D: Healthy shoot formation in cuttings, E: Rooting status of *Prunus cerasifera* rootstock candidates, F: Rooting status of *Prunus persica* rootstock candidates.

Figure 1. Stages of rooting studies

Statistical Analyses

The experiment was conducted as a completely randomised design with 3 replications, each replicate consisting of 20 cuttings. The obtained data were analysed with the statistical programme JMP version 5.0.1. ANOVA was carried out to determine differences between genotypes. The differneces between genotypes were determined with a least significalt differences (LSD) test at 5% significance with the same programme.

Result and Discussion

In terms of percentage rooting of rootstock types, significant differences (P<0.01) that operation highest rooting ratio of the 98.07% P. persica, followed by Myrobolan 29C (96.66%) and GF-677 (95.16%) was found to follow the standard rootstocks. It was determined that rooting rates of rootstock candidates belonging to P. cerasifera and P. domestica colones had similar values and the lowest rooting rate was found in rootstock candidates of P. divaricata colones (74.19%) (Table 1; Figure 1). In the distribution in terms of average root number, it was determined that the highest mean root number was 9.14 and 8.64 in rootstock candidates of P. persica and P. cerasifera species, respectively. The lowest root numbers were found in P. divaricata (3.19) and P. domestica rootstocks (3.44). Referring to the selected control rootstocks, that the average root length maximum root length P. persica is owned rootstocks of candidate species (42.51 mm) P. cerasifera the rootstock of the species, which ranks second in this parameter (40.72 mm), is observed. Root length values were significantly different (P <0.01between all selected and control rootstocks. When viewed as a general kind of rooting for their facilities and selected on the basis of controlling rootstocks, all clones of P. persica, especially in the type of rootstock candidates rooting values have been found quite promising. When we look at the effects of IBA doses on rooting rates in selected and control group rootstocks, it was seen that 2000 mg. L^{-1} dosing has a more positive effect (92.96%) on rooting rate (P <0.01). 3000 mg L^{-1} dosing, where the contrast in effect lower than expected in other applications (69.72%) note appealing, respectively. So, IBA applications gave the best results according to both rooting rates and average root length values which 1000 mg. L -1 and 2000 mg L^{-1} doses (Table 3).

Table 2. Rooting parameters of rootstock candidates on species

Genre Name	Rooting (%)	Average Root Number (Root/Cutting)	Average Root Length (mm)
Prunus persica	98.07 [a]	9.14 [a]	42.51 [a]
Myrobolan-29 C	96.66 [a]	4.55 [c]	29.48 [c]
GF 677	95.16 [a]	4.55 [c]	19.13 [d]
Prunus domestica	88.43 [b]	3.44 [d]	9.56 [f]
Prunus cerasifera	83.54 [bc]	8.64 [b]	40.72 [b]
Pixy	82.54 [c]	3.51 [d]	3.62 [g]
Prunus divaricata	74.19 [c]	3.19 [d]	16.86 [e]
D [0.05]	**5.28 [**]**	**0.34 [**]**	**1.58 [**]**

Differences between averages showed with different letters are statistically important in P<0.05. [**] P <0.01 means [*] P <0.05.

Table 3. Effect of hormone applications on rooting parameters

Hormone Dose (mg.L $^{-1}$)	Rooting (%)	Average Root Number (Piece)	Average Root Length (mm)
0	43.38 ^{d}	2.24 ^{d}	8.86 ^{d}
1000	82.76 ^{b}	5.60 ^{b}	26.31 ^{a}
2000	92.96 ^{a}	6.40 ^{a}	27.28 ^{a}
3000	69.72 ^{c}	4.80 ^{c}	20.46 ^{b}
D $^{0.05}$	**5.75 ****	**0.43 ****	**2.07 ****

Differences between averages showed with different letters are statistically important in P<0.05. ** P <0.01 means * P <0.05.

Table 4 Rooting parameters of selected clone rootstocks.

Number	Clone Name	Rooting (%)	Average Root Number (Root/cutting)	Average Root Length (mm)
1	KL-20	100.00 ^{a}	5.58 ^{h}	50.16 ^{f}
2	KL-12	100.00 ^{a}	3.62 ^{j}	3.72 ^{rs}
3	KL-21	100.00 ^{a}	7.66 ^{f}	52.24 ^{e}
4	KL-24	100.00 ^{a}	13.87 ^{b}	52.25 ^{e}
5	KL-27	100.00 ^{a}	14.91 ^{a}	4.82 ^{qr}
6	KL-23	98.54 ^{abcd}	4.55 ^{I}	31.55 ^{k}
7	KL-1	98.21 ^{bCD}	3.62 ^{j}	6.93 ^{o}
8	MYR-29C	96.66 ^{bcde}	4.55 ^{I}	29.48 ^{l}
9	GF-677	95.16 ^{cdef}	4.55 ^{I}	19.13 ^{n}
10	KL-15	94.41 ^{defg}	9.76	46.23 ^{g}
11	KL-9	94.41 ^{defg}	1.48 ^{l}	1.73 ^{t}
12	KL-2	94.13 ^{defg}	3.51 ^{j}	5.68 ^{pq}
13	KL-59	93.38 ^{defgh}	12.84 ^{c}	64.82 ^{a}
14	KL-11	93.09 ^{defghi}	6.62 ^{g}	54.30 ^{c}
15	KL-61	92.29 ^{defghi}	10.76 ^{d}	58.48 ^{b}
16	KL-3	90.23 ^{efghıj}	2.50 ^{k}	2.66 st
17	KL-43	82.95 ^{hıjklmn}	6.78 ^{g}	54.60 ^{cde}
18	KL-25	88.21 ^{fghıj}	10.76 ^{d}	52.25 ^{in}
19	KL-6	87.16 ^{ghıjk}	4.58 ^{i}	44.04 ^{h}
20	KL-10	85.85 ^{ijkl}	4.55 ^{I}	6.72 ^{p}
21	PIXY	82.75 ^{jklmn}	3.51 ^{j}	3.62 ^{rs}
22	KL-58	79.91 ^{klmn}	6.63 ^{g}	37.77 ^{I}
23	KL-16	79.86 ^{klmn}	6.62 ^{g}	25.34 ^{m}
24	KL-60	79.79 ^{klmn}	4.55 ^{I}	33.63 ^{j}
25	KL-17	79.84 ^{lmn}	10.77 ^{d}	25.45 ^{m}
26	KL-35	77.58 ^{mn}	1.44 ^{l}	1.55 ^{t}
27	KL-8	76.83 ^{mn}	2.48	12.99 ^{o}
28	KL-5	75.83 ^{mn}	6.63 ^{g}	36.81 ^{i}
29	KL-4	72.64no	1.45 ^{l}	1.55 ^{t}
30	KL-63	66.44 ^{o}	3.53 ^{j}	3.74 ^{rs}
31	KL-52	48.87 ^{p}	1.45 ^{l}	6.73 ^{p}
	D $^{0.05}$	**7.48 ****	**0.29 ***	**0.83 ****

Differences between averages showed with different letters are statistically important in P<0.05. ** P <0.01 means * P <0.05.

Considering the rooting rates of selected rootstock candidates, it was seen that 100% rooting success was achieved in six rootstocks (KL-20, KL-12, KL-21, KL-24, KL-27, KL-23) (Table2). It was understood from the table 2 that a rooting success of 70% and above was achieved in almost all of the selected rootstock candidates. The lowest rooting rate was found to be candidate for KL-52 clonal rootstocks. When the rooting rates were analyzed in general, it can be said that all selected rootstocks gave very promising results. Stanica (2007), investigated the effects of perlite and sawdust mixtures on the rooting of six different rootstocks and found that the rooting percentages of 15-60% in the perlite mixture had the best rooting possibilities. Mirabdullbaghi et al. (2011) found that the rooting rates in hybrid rootstocks obtained from *P. domestica* × *P. armeniaca* were between 6.67% and 40.00In another study, Kaur (2015) studied different IBA doses on rooting in *P. persica* and reported that peach rooting rates ranged from 8.5% to 60.56%. Narula (2018) determined the rooting percentage between 12-74% in Kala Amritsari plum using different IBA and PHB doses. Considering many similar studies, it is understood that the rooting rates of Prunus species are between 8-96%. It is seen that rooting rates in most of them are below 60%, some of them are around 6-14%. When we look at the rooting rates of similar studies, it is noteworthy that the rooting results obtained from this study are promising due to 80% of the selected and control rootstocks have a rooting success of 70% and above (Figure 1).

Considering the distribution of root numbers, it was observed that the highest number of roots was obtained from KL-27 (14.91) and KL-24 (13.87) and 6 rootstocks constitute 10-15 roots. It was determined that the lowest number of roots was in KL-35 (1.44) and the average number of roots in 4 rootstocks is around 1.40 (Table 4). When looking at the statistical distribution of root numbers in general, it is understood from the chart that the majority of rootstocks form root number between 1 and 6. Stanica (2007) investigated the

effects of perlite and sawdust mixtures on rooting of six different rootstocks and reported that the average number of roots was 1.8-2%. Mirabdulbaghi et al. (2011) found that the average number of roots in a rootstock study of *P. domestica* x *P. armeniaca* hybrid was quite low compared to our study between which is 1-5%. However, Kaur (2015) found that the average number of roots in peach was quite higher than the results obtained from our study with 20 to 38 and this situation can be caused by the holding time of rooting steels and different hormone doses. As a matter of fact, the average number of roots (10-44) obtained in the rooting study of Narula (2018) in Kala Amritsari plum variety using different IBA and PHB doses were also higher than this study. However, when it is conceived that a low number of the radical study, in different environments and with different types of the formation of such results would be normal line of literature it understood.

It was seen that the highest root length in the distribution of average root lengths of rootstocks used in the study was KL-59 (64.82 mm), followed by KL-61 rootstock with 58.48 mm, and the next six rootstocks were 52.24-54.60 mm in the same group (Table 4). It was understood from the table 4 that the root length spread over a wide range in all rootstocks and the maximum root length distribution was between 2.66-46.23 mm. It was observed that the lowest root length value was in the KL-35 rootstock with 1.55 mm and in 3 rootstocks, they formed a root length of 1.55-2.66 mm in a statistically same group after this rootstock (Table 3-4). Uğur et al. (2016) obtained the highest root length as 172 mm and the lowest root length as 1.33 mm in another study conducted by *P. domestica* plum rootstocks. Stanica (2007) determined the average root length between 2.7-4.8 mm where the effects of perlite and sawdust mixtures on rooting of six different rootstocks were investigated. Mirabdulbaghi et al. (2011) determined the mean root length values between 13.7-18.7mm in a rooting study conducted on a rootstock with *P. domestica* x *P. armeniaca* hybrid. Although these studies are similar to our study, it is noteworthy that the results of Kaur (2015) are slightly higher (100-150 mm). However, the all root length results suggested that, most of the results obtained from our study are compatible with the results in the literature.

Conclusion

With this study, it has been revealed that the natural flora of our country has an important genetic source for rootstocks and stone fruit species. In addition to the changes in climate conditions globally, the breeding of native rootstock candidates which continue to grow healthy in difficult soil conditions is very important for sustainable modern fruit growing. Investigation of rooting capabilities of rootstock candidate genotypes obtained by selection has revealed that these genotypes can give preliminary ideas to the researchers. At the end of the study, a large number of genotypes were selected, some of which showed better rooting characteristics than the rootstocks, which could be promising rootstocks. After obtaining healthy plants from these rootstocks, vegetative reproduction and further study of rootstock features will continue.

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Conflict of Interest

There is no conflict of this manuscript.

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