DOI Link: https://doi.org/10.61586/0g1wQ
Vol.169, Issue.10, Part.1, Oct 2025, PP.58-75

Determination of Sapling Development Performance and Anatomical Analysis of Graft Compatibility in Self-Fertile Almond Variety "Independence" Grafted onto Different Clonal Rootstocks

Deniz Erogul 0000-0001-9559-7855[1*], **Oğuz Dolgun** 0000-0003-0692-0919[2], **Lamiya Quliyeva** 0000-0002-3951-9083, [3], **Ahmet Can Olcay** 0000-0002-2453-1696[4]

[1]Ege University, Faculty of Agriculture, Department of Horticulture, Izmir, Türkiye(*corresponding author); *deniz.erogul@ege.edu.tr*
[2]Adnan Menderes University, Sultanhisar Vocational College Aydin, Türkiye
[3]Azerbaijan State Agricultural University, Department of Plant Protection, Ganja, Azerbaijan
[4]Faculty of Agriculture, Ondokuz Mayıs University, Department of Agricultural Biotechnology,Samsun, Türkiye

Received Feb 2025 Accepted Sep 2025 Published Oct 2025

Abstract

In Türkiye, not only new varieties but also new rootstocks are needed for almond production. In recent years, the availability of novel cultivars has helped with self-fertility and late blooming times, as well as high density and ease of adaptation for machine harvest. There are, however, few studies that examined how these novel cultivars interact with hybrid rootstocks. Before introducing certain new rootstocks to the market, it is necessary to understand the possible "translocated" and "localized" graft incompatibilities.

We determined the sapling development performance and compatibility status of the "Independence" almond variety grafted onto different rootstocks ("GF 677", "Garnem", "Rootpac 20", "Rootpac 40", and "Rootpac R"). The graft success rates of the saplings were high, with the highest rate on Garnem rootstock. The development of the saplings was the highest in June and July. Shoot development and lengths of the saplings grafted onto Garnem rootstock were higher compared to other rootstocks in both years. Saplings grafted onto GF 677 rootstock produced lower values in both years. The thicknesses of the Garnem and Rootpac R rootstocks, with high shoot lengths, were also high.

Considering the compatibility statuses, no significant findings were found in terms of incompatibility among the GF 677 and Independence, Rootpac 40 and Independence, and Garnem and Independence combinations at the examined grafting points. On the other hand, since the cambial continuity was not fully observed in the Rootpac R and Independence, and Rootpac 20 and Independence combinations, the connection of the vascular bundles was not strong, and the necrotic layers preserved their presence in a fragmented form. This circumstance suggested to us that there may be an incompatibility in these combinations.

Keywords: Almond clonal rootstocks, anatomical analysis, grafting incompatibility, *Prunus dulcis*,

1. Introduction

In modern orchard establishments, clone rootstocks that provide higher planting opportunities per unit area and early yielding are used. In terms of sapling production, saplings are obtained between 1 and 3 years by grafting on clone rootstocks. With the use of the right rootstock, growth power can be controlled, and it is possible to obtain high yields with the new rootstocks. Clonal rootstocks shorten the juvenile sterility period of the varieties grafted on them, allowing early fruiting, enabling the production of saplings suitable for changing market demands, affecting the graft compatibility with the variety grafted on it and the development force of the variety, and acting on the flowering time, fruiting time, physiological demands, the constitution of the orchard, fruit yield, and the application way of the cultural practices (Webster, 1995; Rato *et al*., 2008; Morales Alfaro *et al*., 2021). Different studies in *Prunus* indicate that rootstock types affect the performance of the cultivar on which they are grafted (Forcada *et al*., 2012). Rootstocks affect water use efficiency, transpiration, and vegetative growth (Jiménez *et al*., 2013; Opazo *et al*., 2020). Choosing the right rootstock and cultivar combinations is important for optimizing growth and flowering time (Weibel *et al*., 2003).

Almond is one of the most important nut crops in Türkiye (Paizila *et al*., 2022). In 2022, the number of fruit-bearing trees was 13.6 million, and the production amount was 190 thousand tons (TUIK, 2023). In Türkiye, almond cultivation is performed in orchards where rootstock seeds were planted previously. In such orchards, a wide variety of almonds has been formed due to genetic divergence, but the number of registered new varieties and rootstocks is insufficient (Atli *et al*., 2019; Kiliç and Esitken, 2021). In the past few decades, almond seedlings have been the most extensively used rootstock in the Mediterranean region (Rubio Cabetas, 2016). The robust root system of this rootstock is resistant to both drought and limestone. When almond and peach hybrids first appeared, they were initially used on peach trees (*Prunus persica*) before becoming popular for almonds (Reig Cordoba et al., 2019). In recent years, the most popular rootstock in both irrigated and dry areas is the almond and peach hybrid of INRA GF 677 (Rubio Cabetas *et al*., 2017). In particular, in the early 2000s, the hybrid rootstocks obtained by Garnem®, Monegro®, and Felinem® were released to the market, yielding high success and providing nematode tolerance in addition to their red-colored leaves that make them very easy to handle in the nursery (Rubio Cabetas, 2016). GF 677 (*Prunus amygdalus and P. persica*) is one of the most suitable rootstocks for almond and peach, planted on calcareous soils, to overcome lime-induced chlorosis (Spahiu *et al*., 2015). Recently, the "Rootpac" series, from clonal rootstocks with weak growth power, has been developed for almond, peach, nectarine, plum, and apricot. Rootpac 40 is weaker than the Garnem rootstock, and it has been reported that they are suitable for intense planting systems (Jiménez *et al*., 2011).

Across the world, the introduction of novel cultivars and rootstocks has contributed to an increase in almond-planted areas and almond production by improving their agronomic traits, including yield, precocity, and efficiency. In recent years, the availability of novel cultivars has helped the generation of self-fertility, as well as heavy vegetation and ease of adaptation for machine harvest. There are, however, few studies that examined how these novel cultivars interact with hybrid rootstocks (Lordan *et al*., 2019). Under this scenario, the self-fertile almond variety named "Independence" (Independence® (Alm-21 cv.) Patent no. 20295) was developed.

This was accepted as a huge leap for the almond industry, for this recent species was presented as bee-indepented owing to self-fertility and its assumed great capability for free-standing self-pollination (Flore, 2010; Mercer *et al*., 2014; Traynor, 2017).

Before introducing certain novel rootstocks to the market, it is necessary to understand the possible "translocated" and "localized" graft incompatibilities (Reig Cordoba *et al*., 2019). A successful graft union, prolonged survival, and appropriate operation of the grafted plant are all examples of graft compatibility (Goldschmidt, 2014). Numerous intricate biochemical and structural processes, including callus formation, the development of new vascular tissue, and the creation of an active circulatory system across the graft interface, are necessary for the successful construction of a graft (Pina *et al*., 2017). The scion's hormone profile is influenced by its rootstocks (Lordan et al., 2017). Although it is usual to use genetically distinct genotypes, the grafted partners frequently come from the same species or genus. Consequently, graft incompatibility is common, particularly in pairings of interspecies, mostly due to the graft components' dissimilarities in anatomy, morphology, and physiology (Darikova *et al*., 2011).

In almond cultivation, seedling rootstocks have been replaced by GF 677, Garnem, and Rootpac rootstocks in recent years. The "Independence" almond variety is a self-fertile, and studies on sapling development by the referred rootstocks are quite limited. In this study, the rootstock and scion combinations of the "Independence" variety grafted onto "GF 677," "Garnem," "Rootpac 20," "Rootpac 40," and "Rootpac R" rootstocks were investigated in terms of sapling development and histological performance for two growing seasons. Since the use of the "Rootpac" rootstock series is new, there is no preliminary information about the rootstock and scion incompatibilities yet. As "Rootpac 20" is a plum hybrid, "Rootpac 40" is an almond and peach hybrid, and "Rootpac R" is a plum and almond hybrid, symptoms related to possible delayed incompatibilities originating from different species were also investigated.

2. Materials and Method

Rootstocks and varieties used in the experiment

GF 677, Garnem, Rootpac 20, Rootpac 40, and Rootpac R rootstocks were used as rootstock material in the research. The "Independence" almond variety was used as the variety.

GF 677 has been obtained by hybridizing peach (*Prunus persica)* and almond *(Prunus amygdalus*). Garnem rootstock is a hybrid of Garfi almond (*Prunus amygdalus*) and Nemared peach (*Prunus persica*). Rootpac 20 rootstock is a *Prunus besseyi* and *Prunus cerasifera* plum hybrid. Rootpac 40 rootstock is an almond and peach (*Prunus dulcis and Prunus persica*) and (*Prunus dulcis and*

Prunus persica) hybrid. Rootpac R rootstock is a hybrid of plum and almond (*Prunus cerasifera and Prunus dulcis*).

Characteristics of the selected almond variety

Independence® (Alm-21 cv.) almond variety: It requires 400 hours of cooling. It is a self-fertile variety and is harvested in a single shaking of the tree. Its fruits are light, brightly colored, and quite delicious. It blooms at the same time as "Nonpareil" and is harvested 3 days before the "Nonpareil" variety.

Method

In the study, Garnem, GF 677, Rootpac 20, Rootpac 40, and Rootpac R clone rootstocks were supplied by a private company producing saplings with tissue culture method. The rootstocks were planted in pots in the experimental field and then irrigated with drip irrigation method. The scions used in grafting were obtained from saplings planted in the research application orchard in previous years and were stored in polyethylene bags in a cold storage at +4 °C until the spring shoot grafting period. Rootstocks were grafted with T-budding grafting on April 18. Cultural practices such as irrigation, fertilization, and weed control were carried out regularly in the experimental field. Fertilization was accomplished with a drip irrigation system. This study was carried out in two consecutive years at sapling growing area of Parlar Fidancılık Company in Ödemiş district of İzmir province (Türkiye). The saplings samples were initially taken in 1 month after grafting, and two samples were taken from the grafting site of each combination in the following months. The samples were preserved in 70% ethyl alcohol and their anatomical examinations were carried out in laboratories. In the second year, samples were repeatedly taken from each combination and examined again.

Graft-take ratio (%)

Approximately 25 days after grafting, the ratio of the number of buds determined to be live as per the criterion of callus formation in the grafting area to the number of grafts performed was considered, and it provided the graft-take ratio.

Shoot length development (cm)

The shoot length was measured at one-month intervals after the shoot length reached 5 cm in 5 plants selected from the ones subjected to grafting that produced offshoots at each interval. At the end of the growth period, the part from the grafting point to the shoot tip was measured with a meter to determine the shoot length.

Shoot thicknesses development (mm)

In the referred one-month intervals, shoot diameter measurements were made on the middle part of the shoot growing from the grafting point in 5 plants selected from the grafted plants that had formed shoots.

Sapling height (cm)

At the end of the growth period, the height of all saplings in the experiment was measured with a meter, and their average was taken in cm.

Histological studies

Starting from the date of grafting, 2 samples were taken at 30-day intervals by cutting 1 cm below and above the graft point in rootstock and scion combinations and kept in 70% ethyl alcohol until the date of examination. After obtaining the samples with a microtome in different thicknesses according to their strength, they were stained with safranin and methylene blue for better visibility of the tissues. The prepared sections were examined with a microscope and then photographed (Dolgun *et al*., 2008).

Statistical Analyses

The data obtained from the experiment was subjected to analysis of variance using the statistical package program IBM® SPSS® Statistics 22 (IBM SPSS v19, NY, USA). For each on-tree storge, significant differences between the means for each devolepment period were determined by Duncan's multiple range tests at $p \leq 0.05$.

3. Results

Graft success rates

Rootstocks were grafted on April 18 with scions taken from the Independence almond variety using "T-budding." Approximately 25 days after grafting, the graft success rate was determined as the ratio of the number of buds determined to be live as per the criterion of callus formation in the grafting area to the number of grafts performed. The highest graft success rate among the rootstocks was found in the Garnem rootstock with 95%. This was followed by GF 677 rootstock and Rootpac 20 rootstock with 90% graft success rates. The graft success rates for the Rootpac 40 rootstock and the Rootpac R rootstock were 85% and 80%, respectively (Figure 1).

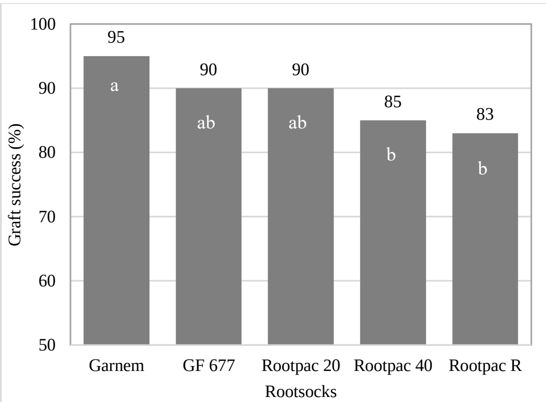

Figure 1. Graft success rates (%)

Shoot length development

Shoot length development of rootstocks grafted with the "Independence" variety in the first year is given in Table 1. Rootpac 40 rootstock showed the lowest shoot length development with 1.11 cm, according to the measurements conducted one month after grafting. This value was 11.37 and 11.87 cm for Garnem and Rootpac R rootstocks, respectively. GF 677 and Rootpac 20 rootstocks showed an intermediate shoot length development with the values of 4.81 cm and 6.00 cm. The most active growth period of the rootstocks was June and July. Shoot length growth slowed down in August and September. Garnem, Rootpac R, and Rootpac 20 rootstocks showed the highest shoot length development rate. Shoot lengths of Garnem, Rootpac R, and Rootpac 20 rootstocks were 63.47 cm, 61.90 cm, and 59.75 cm, respectively. Shoot lengths of Rootpac 40 and GF 677 rootstocks were 43.83 cm and 30.61 cm, respectively.

Table 1. Shoot length (cm) development of rootstocks grafted with the "Independence" variety in the first year

Rootstocks	Development period								
	20th May	8th June	23th June	7th July	23th July	8th August	15th August	8th September	23th September
Garnem	11.37 a[z]**	28.77 b**	34.53 ab**	42.47 a**	51.40 a**	54.55 a**	59.93 a**	61.57 a**	63.47 a**
GF 677	4.81 b	12.96 c	16.84 c	21.33 c	24.68 c	26.10 c	27.82 c	30.61 c	30.61 c
Rootpac R	11.87 a	34.43 a	39.11 a	44.77 a	52.20 a	55.24 a	60.37 a	60.80 a	61.90 a
Rootpac 20	6.00 b	25.90 b	27.54 b	31.83 b	37.00 b	46.35 b	50.15 ab	53.28 b	59.75 ab
Rootpac 40	1.11 c	6.53 d	11.91 d	18.82 c	24.78 c	29.76 c	33.57 bc	35.25 c	43.83 bc

[z] Duncan test was used to determine the differences between the mean data in each column.
**, Significant at $p \leq 0.01$.

Shoot length development of rootstocks grafted with "Independence" variety in the second year is given in Table 2. When the shoot length of the rootstocks grafted with the "Independence" variety was examined at the end of the growing period, the shoot length of the Garnem rootstock was determined as 55.10 cm, and the shoot length of the Rootpac R rootstock was determined as 39 cm. Then the Rootpac 20 rootstock followed with 31.25 cm. The shoot length of the GF 677 rootstock was determined as the shortest, with 23.40 cm, and the shoot length of the Rootpac 40 rootstock was determined as 9.90 cm.

Table 2. Shoot length development of rootstocks grafted with "Independence" variety in the second year

Rootstocks	Development period							
	20th May	8th June	23th June	7th July	23th July	8th August	15th August	8th September
Garnem	8.95 a^{z**}	19.25 a**	26.47 a**	34.25 a**	41.89 a**	47.69 a**	53.24 a**	55.10 a**
GF 677	3.55 b	6.80 bc	9.85 c	14.52 c	17.82 c	19.80 cd	20.15 cd	23.40 c
Rootpac R	7.80 ab	18.85 a	21.20 ab	24.12 b	28.24 b	34.55 b	39.00 b	39.00 b
Rootpac 20	3.50 b	12.56 b	14.85 b	16.89 bc	24.65 bc	27.96 c	29.20 c	31.25 bc
Rootpac 40	1.10 c	4.40 c	6.45 d	7.20 d	7.55 d	8.30 d	9.25 d	9.90 d

^{z} Duncan test was used to determine the differences between the mean data in each column.
**, Significant at $p \leq 0.01$.

Shoot thicknesses

Shoot thicknesses of rootstocks grafted with the "Independence" variety in the first year are given in Table 3. When the shoot thickness of rootstocks grafted with the "Independence" variety was examined, it was determined that the shoot thickness of Rootpac 40 rootstock was the lowest for the first shoots that grew on May 20. The shoot thickness on this rootstock was doubled after 15 days by the beginning of June and reached 3 times its initial thickness by the end of July. While the Garnem rootstock's shoot thickness was 2.03 mm in May, it doubled in July. The shoot thickness of Rootpac R rootstock was initially 2.56 mm, and it was two times thicker by the beginning of August. The shoot thickness of GF 677 and Rootpac 40 rootstocks was the lowest (4.12 mm). The shoot thicknesses of Rootpac R and Garnem rootstocks reached 6.05 mm and 5.60 mm, respectively in the first year.

Table 3. Shoot thickness of rootstocks grafted with the "Independence" variety in the first year

Rootstocks	Development period									
	20th May	8th June	23th June	7th July	23th July	8th August	15th August	8th September	23th September	2nd November
Garnem	2.03^{NS}	2.83^{NS}	3.37^{NS}	3.84^{NS}	4.25^{NS}	4.50^{NS}	4.84^{NS}	5.06^{NS}	5.48 ab^{z*}	5.60 ab*
GF 677	2.56	3.37	3.69	4.07	4.65	4.93	5.34	5.43	6.05 a	6.05 a
Rootpac R	1.81	2.18	2.42	2.68	3.15	3.42	3.67	3.92	4.12 b	4.12 b
Rootpac 20	2.48	2.88	3.16	3.53	3.89	4.08	4.27	4.46	4.92 ab	4.92 ab
Rootpac 40	0.95	1.71	2.03	2.41	2.85	3.09	3.26	3.59	4.13 b	4.13 b

^{z} Duncan test was used to determine the differences between the mean data in each column.
^{NS}, *, Non-significant or significant at $p \leq 0.05$.

When the shoot thickness of rootstocks grafted with the "Independence" variety was examined in the second year, it was observed that the shoot thickness of the Rootpac 40 rootstock was the lowest in the initial shoots that grew on May

20. This value was 1.85 mm for the Rootpac R rootstock and 1.70 mm for the GF 677 rootstock, 1.14 Rootpac 40. (Table 4).

The final shoot thickness was found to be the highest in the Garnem rootstock, with 5.50 mm. The Rootpac R rootstock was found to be the rootstock with the closest thickness to the Garnem rootstock. The shoot thicknesses of the GF 677, Rootpac 20, and Rootpac 40 rootstocks were between 4.28 and 4.03 mm (Table 4).

Table 4. Shoot thickness of rootstocks grafted with the "Independence" variety in the second year

Rootstocks	Development period									
	20th May	8th June	23th June	7th July	23th July	8th August	15th August	8th September	23th September	2nd November
Garnem	2.07 ab[z*]	2.86 a*	3.12 a*	3.62 a*	4.16 a*	4.45 a*	4.52 a*	4.95 a*	5.46 a*	5.50 a*
GF 677	1.85 b	2.54 ab	2.86 b	3.18 bc	3.74 b	3.94 b	4.06 b	4.25 ab	4.81 b	4.81 b
Rootpac R	1.70 bc	2.30 b	2.55 bc	2.84 c	3.12 c	3.54 c	3.75 c	4.05 b	4.22 c	4.22 c
Rootpac 20	2.31 a	2.84 a	3.15 a	3.46 ab	3.71 b	3.87 b	4.05 b	4.12 b	4.28 c	4.28 c
Rootpac 40	1.14 c	1.75 c	2.16 c	2.43 d	2.89 d	3.14 d	3.32 d	3.61 c	4.03 d	4.03 d

[z] Duncan test was used to determine the differences between the mean data in each column.
*, Significant at $p \leq 0.05$.

Sapling heights

Sapling heights found as the result of grafting by the "Independence" variety were 78.47 cm in Garnem rootstock, 74.75 cm in Rootpac 20 rootstock, 58.83 cm in Rootpac 40 rootstock, 76.90 cm in Rootpac R rootstock, and 45.60 cm in GF-677 rootstock in descending order (Figure 2).

At the end of the second year, Garnem rootstock grafted with the "Independence" almond variety indicated the highest sapling height with 134.67 cm (Figure 2). Then the Rootpac R rootstock followed the Garnem rootstock with a height of 113.96 cm. The variety grafted onto Rootpac 40 and Rootpac 20 rootstocks provided heights of 92.11 cm and 87.65 cm, respectively. The "Independence" almond variety grafted onto GF 677 rootstock provided the lowest height with 69.2 cm (Figure 2).

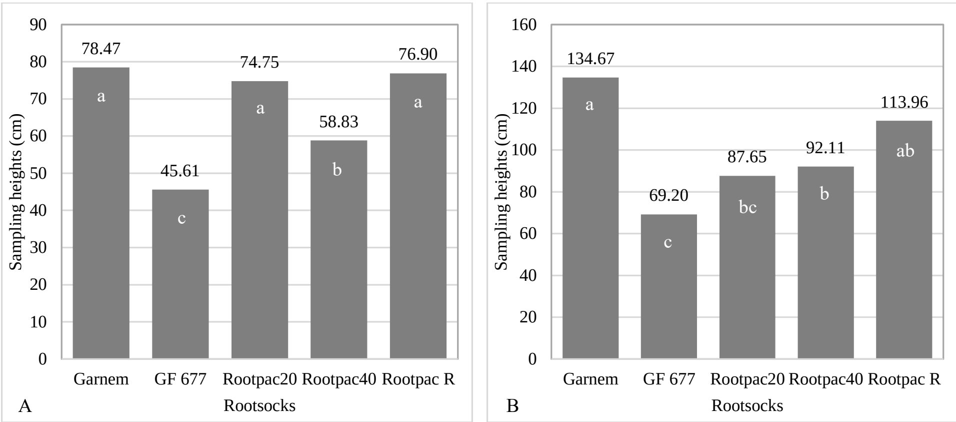

Figure 2. Sapling heights of rootstocks grafted with the "Independence" variety in the first (A) and second (B) year

Histological studies

In plants that can be propagated by grafting, a good graft union results in quality saplings that grow in a short time. Consideration of the tissue development after grafting by the samples taken from the grafting area at periodic intervals can provide some preliminary information on the success of the graft. The main facts in graft union are the cohesion of the rootstock and scion, the formation and development of callus cells, the connection of the cambium of the rootstock and scion developing within the callus, and the formation of vascular continuity. After grafting, large parenchyma cell callus tissue is formed, which the graft elements begin to produce in order to close the wound after the injury in the cut areas. With the help of this tissue, the first connection is established between two plant parts. In this way, some water exchange is provided, albeit limited. However, this situation is not permanent. Callus provides the first contact and partial water intake, as well as filling the gaps between the graft elements.

Necrotic plaques, which are formed by dead cells and seen in graft samples, are a layer of dried, crushed, and eventually dead cells on the cut surfaces of both the rootstock and the scion or the graft bud. The necrotic plaque serves to protect the injured tissue from pathogens and to reduce water loss. In most plants, callus tissue is formed by the division of the vascular cambium, by the differentiation of cells, or by the differentiation of some cells between xylem and phloem cells. As the new callus increases in volume, it ruptures the necrotic plate and begins to expand into the spaces between the rootstock and the scion/bud. The new vascular cambium begins to form from the vascular cambium of the rootstock and scion inwards or with the differentiation of some cells within the callus into the cambium. In the later stages, the new vascular cambium cells begin to divide and begin to produce xylem tissues inwards and phloem tissues outwards. Regeneration and bridging of xylem and phloem, called conducting tissues, provide transport of water and assimilates along the rootstock and scion, and the tight interlocking of new xylem fibers provides mechanical strength.

In the study, the "Independence" variety was grafted onto Rootpac R, Rootpac 20, Rootpac 40, Garnem, and GF 677 rootstocks, and as explained in the material and method section, the developmental strengths of rootstock and scion after grafting and the histological development of graft union were observed in the sections prepared from the samples taken at periodic intervals. In all combinations, especially in the early period samples, fragmentation, dispersion, and breakage were observed in the samples when the sections were taken with the microtome. Therefore, since the staining process could not be done, the sections were examined without staining. In the present study, the histological examinations were carried out to determine the development of graft union and possible incompatibility symptoms in the "Independence" variety grafted onto rootstocks with different developmental strengths.

30th-day samples

In the 30th-day samples, it was not possible to take the whole section from most of the samples in all combinations due to the weak connection between the rootstock and the scion. In the sections, a limited but satisfactory callus formation, which is the first step of wound repair after grafting, was observed in all combinations (Figure 3). However, a strong callus formation and connection was not observed. Necrotic layers were observed as very dark, distinct piles. No development was observed regarding cambial differentiation.

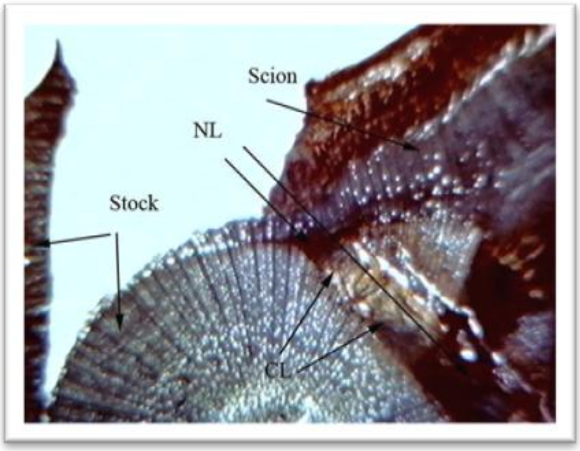

Figure 3. GF 677 rootstock / Independence variety combination (NL: necrotic layers)

60th-day samples

In the sections taken from GF 677 and Independence, Rootpac 40 and Independence, and Garnem and Independence combinations, intense cellular activity was observed on both the rootstock and scion sides of the combinations, and cellular activity was observed not only for callus but also for cambial differentiation in all sections; cell divisions and developments were observed in the cortex region of rootstock and scion, within the callus tissue and at the edges of cambium tissues. It was observed that necrotic layers were broken down and absorbed by the strongly developing callus, and in some cases, they maintained their presence as thin strips on the cross-sectional surfaces of rootstock and scion. Although cambium development and connection could not be observed very clearly, xylem and phloem formations were observed within the callus tissue.

In the Rootpac 20 and Independence, and Rootpac R and Independence combinations, similar callus formations were observed in the rootstock and scion (Figure 4).

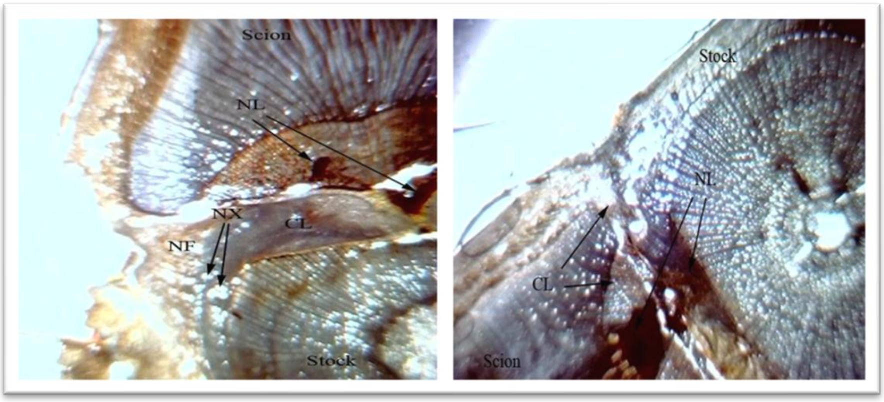

Figure 4. Rootpac 20 rootstock and Independence variety combination (left photo); Rootpac R rootstock and Independence variety combination (right photo) (CL: callus, NX: new xylem, NF: new phloem, NL: necrotic layers)

Cambial continuity was not clearly visible. Xylem and phloem formations were observed in places within the callus. Necrotic layers clearly maintained their presence.

90th-day samples

In the sections taken from GF 677 and Independence, Rootpac 40 and Independence, and Garnem and Independence combinations, intense cellular activity was observed on both the rootstock and scion sides of the combinations, and cellular activity was observed not only for callus but also for cambial differentiation in all sections; cell divisions and developments were observed in the cortex region of rootstock and scion, within the callus tissue and at the edges of cambium tissues. It was observed that necrotic layers were broken down and absorbed by the strongly developing callus, and in some cases, they maintained their presence as thin strips on the cross-sectional surfaces of rootstock and scion. Although cambium development and connection could not be observed very clearly, xylem and phloem formations were observed within the callus tissue (Figure 5).

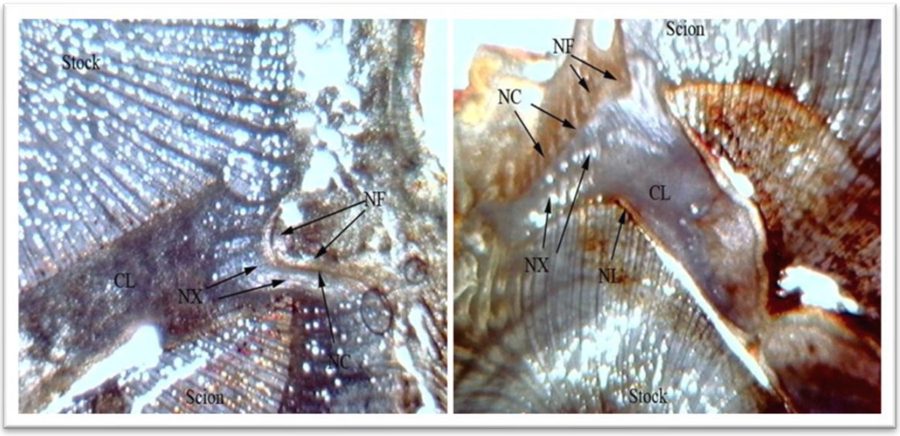

Figure 5. GF 677 rootstock and Independence variety combination (left photo); Garnem rootstock and Independence variety combination (right photo) (CL: callus, NX: new xylem, NF: new phloem, NL: necrotic layers)

Xylem and phloem cell clusters, which were vascular cells, were observed as stripes along the graft line. The cambial connection was wavy due to the intense development of callus tissue. Cambial continuity was not fully observed in the Rootpac 20 and Independence, and Rootpac R and Independence combinations, and the existing ones showed a problematic development.

120th-day samples

In the 120th-day samples and in the sections prepared from the samples taken in the following periods, it was observed that the callus tissue generally filled the gaps between the rootstock and the scion. Xylem and phloem tissues were clearly visible, and the xylem and phloem tissues were successfully bridged (Figure 6).

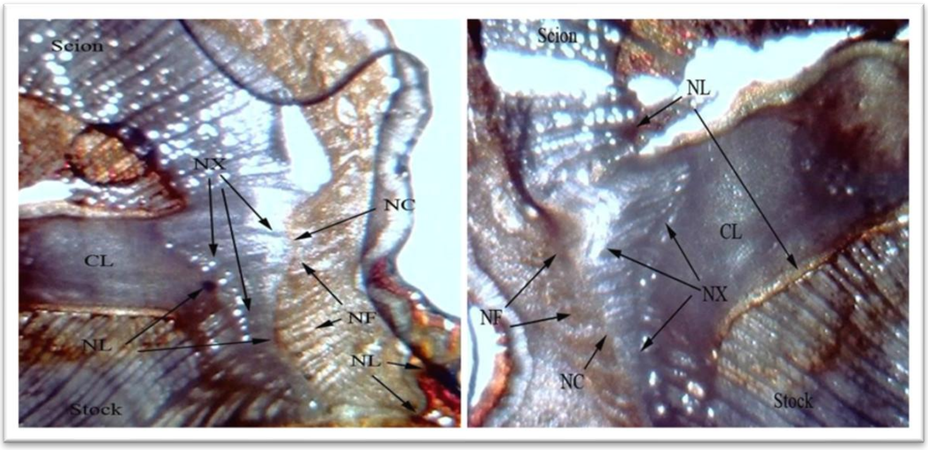

Figure 6. Rootpac 40 rootstock and Independence variety combination (left photo); GF 677 rootstock and Independence variety combination (right photo) (CL: callus, NX: new xylem, NF: new phloem, NL: necrotic layers)

Cambial continuity was achieved successfully and formed transmission bundles. On the other hand, cambial continuity was problematic in the Rootpac R and Rootpac 20 combinations. Xylem and phloem formations were present but weak. Necrotic layers preserved their presence.

4. Discussion and conclusions

Graft success rates of the "Independence" almond variety grafted onto different rootstocks were found to be high. The highest success rate among the tested rootstocks was in the Garnem rootstock, with 95%. GF 677 and Rootpac 20 rootstocks followed it with a 90% graft success rate. The Rootpac 40's graft success rate was determined to be 85%, Rootpac R graft success rate was 80%. In the grafting studies of Ugur *et al*. (2024), including 37 rootstocks and two almond varieties (Feragnes and Ferraduel), the grafting success rates ranged from 57.7 to 96.0%. The highest and the lowest grafting success rates were 96.0% and 58.8%, with the average success rate being 73.2%.

The shoot development and shoot lengths provided by the "Independence" variety grafted onto Garnem rootstock were found to be higher for both years compared to other rootstocks. The GF 677 and Rootpac 40 rootstocks had lower values in both years. While the shoot length of Rootpac R rootstock was 61.9 cm in the first year, its shoot development was determined to be 39 cm in the second year, and thus it developed rapidly in the first year, and a pause was observed in its development in the second year. While Rootpac R showed regular growth in the first year, cracks occurred in the sapling trunk when the scion part started to grow.

Garnem and Rootpac R rootstocks indicated shoot lengths of 63.47 cm and 61.90 cm, respectively, and Rootpac 20 rootstock indicated a growth power close to these rootstocks with a shoot length of 59.75 cm. Rootpac 40 rootstock had a much lower shoot length than all the other rootstocks in the beginning, but it indicated rapid growth in July and had a shoot length greater than GF 677 rootstock.

In the second year, the growth power of the rootstocks decreased. The rootstock with the least growth power was Rootpac 40, and it had a shoot length of 43.83 cm in the first year, while it decreased by 75% to 9.90 cm in the second year. Garnem rootstock maintained its strong growth, and its shoot length was 55.10 cm, which decreased by 11% compared to the first year. Rootpac R rootstock was 61.90 cm in the first year and 39 cm in the second year, and its growth decreased by 37%. Rootpac 20 rootstock was 59.75 cm in the first year and 31.25 cm in the second year, and GF 677 was 30.61 cm in the first year and 23.40 cm in the second year, with decreases in growth of 44% and 23%, respectively.

Garnem rootstock grafted with the "Independence" variety indicated strong shoot growth in both years. Rootpac 40 and GF 677 rootstocks indicated weaker growth, while the Rootpac 40 rootstock had the weakest growth in the second year.

It was determined that the rootstocks with high shoot lengths in the first year also had high shoot thicknesses. The thicknesses of Garnem and Rootpac R rootstocks with high shoot lengths were also high. While the shoot length of Garnem rootstock was high in the second year, it produced the highest shoot thicknesses.

The development in shoot length was parallel to the development in shoot thickness. Shoot lengths of Garnem, Rootpac R, and Rootpac 20 rootstocks were higher than Rootpac 40 and GF677 rootstocks, while shoot thicknesses were also higher than these rootstocks. The rootstocks with strong shoot length increase also had thicker shoots.

Kilic and Esitken (2021) reported the shoot lengths as 18.82-8.27 cm and the shoot diameter as 0.72-0.20 cm in one-year-old shoots in the Sarayönü district of Konya Province (Türkiye, central Anatolia, arid climate) in 2020-2021. Lordan *et al*. (2019) assessed the performance of Cadaman®, Garnem®, INRA GF 677, IRTA-1, IRTA-2, Ishtara®, Adesoto, Rootpac® 20, Rootpac® 40, and Rootpac® R rootstocks with two promising almond cultivars (Marinada and Vairo). Bloom and nut ripening dates were affected by rootstock genotype. Both "Marinada" and "Vairo" cultivars indicated low biennial bearing, with some differences among rootstocks, with IRTA-2 and Adesoto providing the lowest values. Garnem® provided the biggest trees. Rootpac® 20 was the most dwarf rootstock, followed by IRTA-1, Adesoto, Ishtara®, Rootpac® R, and Rootpac® 40. Differences in tree volume and vigor of these rootstocks suggested that INRA GF 677 would be a suitable rootstock for low- to medium-density plantings with wide spacing, whereas Rootpac® R and IRTA-1 would be suitable rootstocks for medium- and high-density plantings. In a study involving five almond cultivars (‘Tuono’, ‘Lauranne’, ‘Soleta’, ‘Belona’, and ‘Guara’) grafted onto three rootstocks

('Garnem', 'Rootpac-40', and 'Rootpac-20'), 'Garnem' and 'Rootpac-40' were identified as the most vigorous and moderately vigorous rootstocks, respectively. Saplings grafted onto 'Garnem' exhibited the highest trunk cross-sectional area (TCSA) values, while those grafted onto 'Rootpac-20' displayed the most pronounced dwarf phenotype (Yahmed et al., 2016).

In the combinations where sections were taken to examine graft incompatibility, sections could not be taken as a whole, therefore, thicker sections were taken to ensure integrity. In thicker sections, it was difficult to examine the tissues under the microscope. Most of the sections taken with the microtome were fragmented and dispersed during the transportation or staining process, however, the sections that were able to partially preserve their integrity were examined under the microscope and photographed without being subjected to the staining process. In the sections that could be examined, no clear finding of incompatibility was encountered in the GF 677 and Independence, Rootpac 40 and Independence, and Garnem and Independence combinations. On the other hand, there may be incompatibility in the Rootpac R and Independence, and Rootpac 20 and Independence combinations as cambial continuity was not fully observed. The connection of the vascular bundles was not observed to be strong, and the necrotic layers maintained their presence in a fragmented form.

The fragmentation of the sections obtained from all the combinations taken, even in the later periods of the study, suggested that this may be a result of the weak connection of callus and vascular bundle, which was due to the different developmental strengths of the rootstock and scion.

In general, successful grafting occurs due to the formation of necrotic tissues in the cuttings after grafting, the formation of callus tissue as a response to wounding on the rootstock and scion sides, the binding of callus tissues, the absorption of necrotic layers on the callus side and their decrease or elimination, the formation of cambium cells developing both from within the callus and from the parts close to the cut surfaces of the grafting elements and the provision of cambial bridging, and finally, the successful bridging of xylem and phloem tissues, which are the vascular bundles. The presence of several of these can be considered as an indicator of incompatibility (Sitarek *et al*., 2006; Pina and Errea, 2008). In our study, a similar situation was encountered in the Rootpac R and Independence and Rootpac 20 and Independence combinations.

Necrotic tissues were densely formed as a result of cell deaths in the cut areas that occurred during grafting. Necrotic tissue densities were found at different levels in the sections obtained periodically from the samples of all combinations, which suggested differences in the grafting process and the weak connections of the grafting areas. Shortly after grafting, callus tissue began to develop in the cambium tissues located at the edges of the cut areas of the rootstock and scion as a response to wounding. This developed over time, filled the gaps partially/completely, and absorbed the necrotic layers partially or completely in time. It was observed that the callus tissue partially/completely filled the gaps in the cut areas in the sections taken from the combinations in different periods and absorbed the necrotic layers as observed in the sections.

In grafting, the connections between two plant parts are formed by the tight bridging of xylem and phloem tissues formed by the cambium tissue. The weakness of this connection plays an important role in the transport of water and nutrients. In cases of weak connections, inadequate development, and shoot death are observed in the graft combinations. In addition, the weakness of the vascular connection is considered as a sign of incompatibility (Dolgun *et a*l., 2008). In our study, no significant incompatibility was found in the GF and Independence, Rootpac 40 and Independence, and Garnem and Independence combinations. However, incompatibility signs were observed in the Rootpac R and Independence and Rootpac 20 and Independence combinations.

At the end of both years, the saplings of the "Independence" variety grafted onto Garnem rootstock had the highest height, with 134.57 cm. This sapling was followed by the Rootpac R rootstock with 113.96 cm. The height of the saplings of the "Independence" variety grafted onto Rootpac 20 and Rootpac 40 rootstocks indicated a semi-dwarf structure. The ones grafted onto GF677 rootstock had the lowest growth power with, 69.20 cm.

The highest levels of plant growth were observed in June and July, while growth rates began to decline in August.

Shoot length measurements of the rootstocks grafted with the Independence cultivar revealed that Rootpac40 and GF677 exhibited the weakest growth in the first year, whereas Garnem, RootpacR, and Rootpac20 showed more vigorous development. In the second year, Rootpac40 displayed the least growth with 9.90 cm, followed by GF677 with a shoot length of 23.40 cm. Garnem demonstrated strong shoot growth, reaching 55.10 cm. Shoots with greater length also had greater thickness, indicating a parallel relationship between shoot length and thickness.

Regarding the seedling height of the rootstocks grafted with the Independence cultivar, Garnem and RootpacR produced taller seedlings compared to the others, while seedlings grafted onto GF677, Rootpac40, and Rootpac20 showed weaker growth.

Acknowledgements

We would like to thank the Ege University Scientific Research Projects Commission (GAP-2020-21871) for supporting this project and enabling its implementation.

References

Atli H.S., Can C., Baş M., 2019. Nematode-resistant, clonal almond rootstock breeding by crossing in Turkey. *Turkish Journal of Agriculture and Forestry*, 43(4): 389-394. https://doi.org/10.3906/tar-1805-152

Darikova J.A., Savva Y.V., Vaganov E.A., Grachev A.M., Kuznetsova G.V., 2011. Grafts of woody plants and the problem of incompatibility between scion and rootstock (a review). Journal of Siberian Federal University, *Biology* 1(4) : 54-63. http://elib.sfu-kras.ru/bitstream/handle/2311/2376/Darikova.pdf;jsessionid=EBDD19E12C184FFEB7C0654900E56F48?sequence=1

Dolgun O,. Tekintas F.E., Ertan E., 2008. A histological investigation on graft formation of some nectarine cultivars grafted on pixy rootstock. *World Journal of Agricultural Scien*ces 4(5): 565-568.

Flore A., 2010. Self-pollinating almonds key to bountiful harvest. *Agricultural Research* – USDA .

Forcada C.F., Gogorcena Y., Moreno M.A., 2012. Agronomical and fruit quality traits of two peach cultivars on peach-almond hybrid rootstocks growing on Mediterranean conditions. *Scientia Horticulturae*, 140: 157-163.

Goldschmidt E.E., 2014. Plant grafting: new mechanisms, evolutionary implications. *Frontiers in Plant Science*, 5(727)1-9.

Jiménez S., Pinochet J., Romero R., Gogorcena Y., Moreno M.A., Espada J.L., 2011. Performance of peach and plum based rootstocks of different vigour on a late peach cultivar in replant and calcareous conditions. *Scientia Horticulturae*, 129: 58-63.

Jiménez S., Dridi J., Gutiérrez D., Moret D., Irigoyen J.J., Moreno M.A., Gogorcena Y., 2013. Physiological, biochemical and molecular responses in four *Prunus* rootstocks submitted to drought stress. *Tree Physiology* 33(10):1061-1075.

Kilic M., Esitken A., 2021. Rootstock selection in almond (*Prunus amygdalus*) seedling population at Gozlu. *Agricultural Enterprise Journal of Bahri Dagdas Crop Research* 10(2): 190-198.

Lordan J., Fazio G., Francescatto P., Robinson T., 2017. Effects of apple (*Malus* × *domestica*) rootstocks on scion performance and hormone concentration. *Scientia Horticulturae*, 225: 96-105.

Lordan J., Zazurca L., Maldonado M., Torguet L., Alegre S., Miarnau X., 2019. Horticultural performance of 'Marinada'and 'Vairo'almond cultivars grown on a genetically diverse set of rootstocks. *Scientia Horticulturae*, 256 ,108558.

Mercer D., Onulak J., Fagan J.M., 2014. Alternative pollination methods for California almond trees in response to colony collapse disorder.

Morales Alfaro J.,, Bermejoc, A., Navarro P., Oliver A.Q., Salvador, A., 2021. Effect of Rootstock on Citrus Fruit Quality: A Review. *Food Reviews International* 9(5): 2835-2853.

Opazo I., Toro G., Salvatierra A., Pastenes C., Pimentel P., 2020. Rootstocks modulate the physiology and growth responses to water deficit and long-term recovery in grafted stone fruit trees. *Agricultural Water Management*, 228:105897.

Paizila A., Karcı H., Motalebipour E.Z., Güney M., Kafkas S.,2022. Quantitative trait loci analysis for flower-related traits in almond (*Prunus dulcis*). Pl*ant Breeding,* 141(1): 119-132.

Pina A., Errea P., 2008. Influence of graft incompatibility on gene expression and enzymatic activity of UDP-glucose pyrophosphorylase. *Plant Science,* 174(5): 502-509.

Pina A., CookSon S.J., Calatayud A., Trinchera A., Errea P., 2017. Physiological and molecular mechanisms underlying graft compatibility.

In Vegetable grafting: principles and practices, Wallingford UK: CABI: 132-154.
Rato, A.E., Agulheiro, A.C., Barraso J.M., Riquelme, F., 2008. Soil and rootstock influence on fruit quality of plums (*Prunus domestica* L.). *Scientia Horticulturae*, 118: 218-222.
Rubio Cabetas M.J., 2016. Almond rootstocks overview. *Option Mediterraneennes A*, 119: 133-143.
Reig Cordoba G., Salazar González Á. E., Zarrouk O., Font i Forcada C., Val Falcón J., Moreno Sánchez, M.A., 2019. Long-term graft compatibility study of peach-almond hybrid and plum based rootstocks budded with European and Japanese plums. *Scientia Horticulturae,* 243, 3: 392-400.
Rubio-Cabetas M.J., Felipe A.J., Reighard G.L., 2017. "Rootstock development. In Almonds: botany, production and uses" Wallingford UK: Cabi : 209-227.
Sitarek M., 2006. Incompatibility problems in sweet cherry trees on dwarfing rootstocks. *Latvian Journal of Agronomy*, 9: 140-145.
Spahiu E., Rama P., Hodaj B., 2015. Callus Induction and Adventitious Shoot Regeneration from Different Explants of Rootstocks GF-677 (*Prunus amygdalus x P. persica*). *Albanian Journal of Agricultural Sciences*,14(1): 36.
TUIK, 2023. Turkish Statistical Institute. https://biruni.tuik.gov.tr.
Traynor, J.A., 2017. A history of almond pollination in California. Bee World 94, 69-79.
Ugur R., Saridas M.A., Atli H.S., 2024. Investigation of graft compatibility rates of different clonal rootstocks with some peach and almond varieties. In proceedıngs of VI. International Agricultural, Biological, Life Science Conference, *AGBIOL* - 517.
Webster, A.D., 1995. Rootstock and interstock effects on deciduous fruit tree vigour, precocity, and yield productivity. *New Zealand Journal of Crop and Horticultural Science*, 23: 373-382.
Weibel A., Johnson S., DeJong M., 2003. Comparative Vegetative Growth Responses of Two Peach Cultivars Grown on Size-controlling Versus Standard Rootstocks. *Journal of the American Society for Horticultural Science* 128: 463-471.
Yahmed, J.B., Ghrab, M., Mimoun, M.B. 2016. Eco-physiological evaluation of different scion-rootstock combinations of almond grown in Mediterranean conditions. *Fruits*, 71(3), 185-193.