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Anatomical Analysis of Graft Compatibility in Some Almond Scion-Rootstock Combinations

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ABSTRACT

In this study, graft compatibility between almond cultivar Lauranne and almond seedling and Rootpac R, Rootpac 90, Rootpac 70 and Rootpac 40 clonal rootstocks was anatomically investigated. The anatomical analysis of scion/rootstock combinations was performed by taking cross sections for 30 days and 12 months after T-budding in June, 2017. It was determined that, 30 days after grafting, the callus cells developed but cambial continuity has not occurred between the rootstock and scion tissues in all scion/rootstock combinations. 12 months after grafting, cambial relation was established, vascular differentiation was observed, regular parenchymatic tissue properties and scleroid (petrosal cell) cells and sclerenchyma bundles were seen in the graft union. There was no problem in terms of rootstock- scion compatibility in Lauranne almond cultivar which was grafted on almond seedling and some Rootpac clonal rootstocks.

Keywords: *Prunus dulcis*; Grafting; Almond seedling; Rootpac clonal rootstocks

1. Introduction

Fruits have gained more interest in recently due to high nutritional value, phytochemical content and human health effects. Fruit has been recognized as a good source of vitamins and minerals, and for their role in preventing vitamin C and vitamin A deficiencies (Kamiloğlu et al 2009; Tosun et al 2009; Zorenc et al 2016; Çalışkan et al 2017). Almond has a high nutritional value, and is used as raw material in many sectors including food, cosmetics and pharmaceutical industry (Gebauer et al 2016; Taş & Gökmen 2017). Cultivation and trade of the almond around the world is significant.

The most important factor limiting the cultivation of almond in the world is special ecological demands. In addition to this, factors such as high lime rate of soil and some soil-based diseases also play a limiting role in almond cultivation. Problems arising from different soil types, climatic conditions, diseases and damages that restrict cultivation can be eliminated by using suitable rootstocks. Thus, the need for rootstock to overcome these limitations is essential for growing stone fruit, including almonds, in many regions of the Mediterranean basin (Felipe 1989). Proper rootstocks must be used in order to obtain high quality and yield in different climatic and adverse soil conditions, to control the growth vigour of trees, to provide early tolerance for infestation and resistance against diseases and pests. There are also approaches to increase yield by applying frequent plantation methods which allow more trees to be kept in unit area in modern cultivation. For this reason, it is very important to use grafted cultivars on suitable rootstocks in commercial fruit growing. Factors such as technical errors during grafting, time of grafting, selection of materials to be used in grafting and some virus diseases affect grafting success. The main factor that determines the success of grafting is compatibility status between the rootstock and cultivar. Almond seedlings are traditionally used for almond rootstock. In addition, peaches, plums and apricot seeds are also used as rootstock. In addition to studies on seedling rootstocks, clonal rootstock studies in almond are also widely carried out. Peach x almond hybrids (GF 557, GF 677, Hansen 2168, Hansen 536 etc.) (Özcan 2000), plum clones (Marianna GF 8-1, Marianna 2624 etc.), plum x peach x almond hybrids (Garnem etc.) (Atlı et al 2011) have become widespread in recent years. Many studies have been conducted on scion-rootstock compatibility between some almond cultivars and almond rootstocks. It has been reported that, graft compatibility with almond is highly variable; hence, preliminary trials are required before a particular combination of almond is utilized on plum (Rubio-Cabetas et al 2017). Considering their performance against soil-borne problems such as chlorosis or nematode, it is suggested to increase seedling production with clone rootstocks of GF 677, Garnem, Cadaman and Rootpac series instead of seedling rootstocks for modern almond cultivation (Arquero et al 2002; Felipe 2009; Bielsa et al 2015; Rubio-Cabetas 2015). In addition there are several new clones also commercially propagated, such as ‘Replantpac’ (Rootpac-R), a myrobalan x almond hybrid with compatibility with almond (Pinochet 2010). Although without prior extensive field evaluation, dwarfing rootstocks such as Rootpac 20, Rootpac 40, Rootpac 70 and Rootpac 90 were released and considered for almond (Rubio-Cabetas et al 2017). It is very important that incompatibility status of almond cultivars to be grafted on a large number of rootstocks with various characteristics are known before the orchard is established. The incompatibilities that will arise after the orchard plantation will cause serious economic losses. For this reason, anatomical examination of the coexistence of different rootstock-type graft combinations is a rescuer approach.

Graft incompatibility situations between different new rootstocks and almond cultivars should be evaluated. Rootpac clonal rootstocks have superior properties and also some of them allow intensive almond plantation in recent years. Until now, some of the almond cultivars on these rootstocks have not been assessed anatomically in terms of graft compatibility.

In this study, anatomical investigation of graft compatibility performance of Laurenne almond cultivar on almond seedling and clonal rootstocks (Rootpac 40, Rootpac 70, Rootpac 90 and Rootpac R) was aimed. Formation in the graft union was anatomically examined in the samples taken 30 days and 12 months after budding.

2. Material and Methods

2.1. Plant materials

In the study, five different rootstocks (almond seedling, Rootpac R, Rootpac 90, Rootpac 70, Rootpac 40) and Lauranne cultivar were used as plant material. Almond seedlings are used as control rootstock. The research was carried out in the greenhouse conditions of Gaziantep Pistachio Research Institute. Clonal rootstocks were grown by tissue culture and obtained from a commercial company, and were stuck in tubes of 18x32 cm size filled with soil, burnt stallion and peat mortar in a ratio of 1: 1: 1. One year old rootstocks, that reached the grafting thickness, were done T-budding in June 2017 period. Laurenne cultivars as scions are prepared from annual exiles from the trees in the collection parcel of Gaziantep Pistachio Research Institute.

Lauranne, a self fertile and highly productive cultivar which blooms late, is resistant to cold weather and tolerant to fungal diseases.

'Replantpac' (Rootpac® R) is a new plum-almond hybrid selected by Agromillora Iberia, S.L., Barcelona, Spain, for use mainly as a rootstock for Japanese plum (*Prunus salicina* Lindl.), peach, and nectarine [*P. persica* (L.) Batsch] cultivars, but it can also be used for almond [*P. dulcis* (Mill.) D. A. Webb, syn. *P. amygdalus* Batsch] and some apricot (*P. armeniaca* L.) cultivars (Pinochet 2009).

Rootpac R (*P. cerasifera x P. dulcis*): It is a rootstock that is compatible with cultivars of peach, nectarine, plum, almond and apricot, able to withstand hard soil conditions. It is highly productive and tolerant to asphyxia, highly tolerant to chlorosis and root-knot nematodes (Jiménez et al 2013).

Rootpac 90 (*P. persica x P. davidiana) x (P. dulcis x P. persica*): It is a new rootstock that is well adapted to intensive production conditions, and produces fruit with high yield and good quality. It is more productive than GF 677 and Garnem. It adapts very well to all climate conditions, both warm and colder climates. It is sensitive to asphyxia. It is highly tolerant to chlorosis. It is moderately resistant to root-knot nematodes (Pinochet 2009).

Rootpac 70 (*P. persica x P. davidiana) x (P. dulcis x P. persica*): It is a new rootstock suitable for intensive plantations, reducing the cost of production with good quality of fruit and early harvest. It adapts very well to all climate conditions, but particularly to warm conditions (low chilling areas). It is sensitive to asphyxia and salinity. It is tolerant to chlorosis. It is moderately resistant to root-knot nematode (Jiménez et al 2011).

Rootpac 40 *(P. dulcis x P. persica*) *x (P. dulcis x P. persica*): It is a new rootstock suitable for intensive plantations, with good fruit quality and early harvesting properties. It is extremely well adapted to the most warm production conditions and especially recommended for cultivars of peach, nectarine, almond and some Japanese plum. It is more tolerant to asphyxia than most peach and peach x almond hybrids and is moderately tolerant to chlorosis, salinity and root-knot nematodes (Yahmed et al 2016).

Almond seedling: It is a traditional rootstock widely used in almond cultivation. The compatibility with almond cultivars is high. The almond seedlings are suitable for calcareous, dry and gravelly soils and increase the growth vigor of the cultivar grafted on it (Yılmaz 2010).

The growth vigor of Rootpac R, Rootpac 90 and almond seedling rootstocks is high while that of Rootpac 70 and Rootpac 40 are moderate.

2.2. Methods

The rootstocks used in the study were grafted with the Laurenne cultivar by T budding method in June, 2017. 30 days and 12 months after budding, budded samples from each combination were taken from 5 cm below and above the graft union (Figure 1) and were stored in FAA solution until the sections were taken. Cross sections, 20-25 microns thick, were taken from the graft union by hand, stained using the safranin dyeing method and observed under microscope (Seferoğlu 1991).

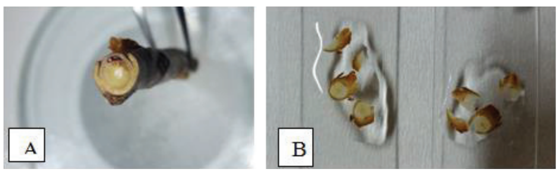


Figure 1- View of grafted union 5 cm above (A) and transversal cuts of *Prunus dulcis* (B)

3. Results and Discussion

3.1. 30 days after grafting

It was determined that, callus cells developed but cambial differentiation between rootstock and scion tissues has not begun in the sections budded onto seedling, Rootpac R and Rootpac 70 rootstocks

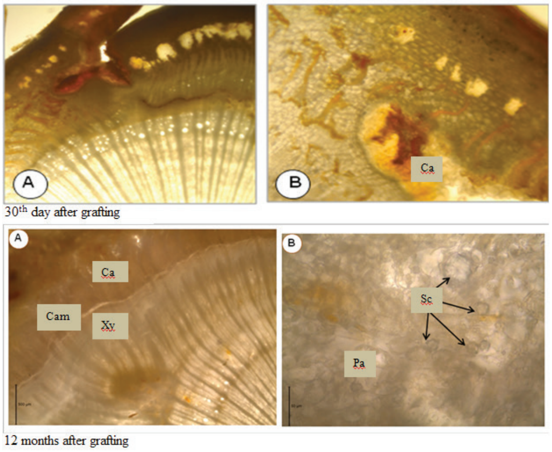


Figure 2- Cross sections taken 30 days and 12 months after the budding from budded parts of Laurenne cultivar on almond seedling; A, callus cells (Ca), cambium (Cam) and new xylem elements (Xy); B, parenchymatic cells (Pa), sclereids (Sc)

(Figure 2, Figure 3, Figure 4). Similarly, too many callus cells developed but cambial continuity between rootstock and scion tissues has not been established yet in the sections budded onto Rootpac 90 and Rootpac 40 rootstocks (Figure 5, Figure 6).

3.2. 12 months after grafting

In the cross sections taken 12 months after budding of Laurenne onto seedling rootstock, budding formation was found successful, cambial continuity sustained, new transmission tissues formed from the cambium, callus cells gained regular parenchymatic tissue properties and numerous sclereid cells were encountered in the parenchymal cells (Figure 2).

In the cross sections taken from the budded parts on Rootpac R rootstock, it was observed that the callus cells filled the callus completely between the budding elements, gained regular parenchymatic

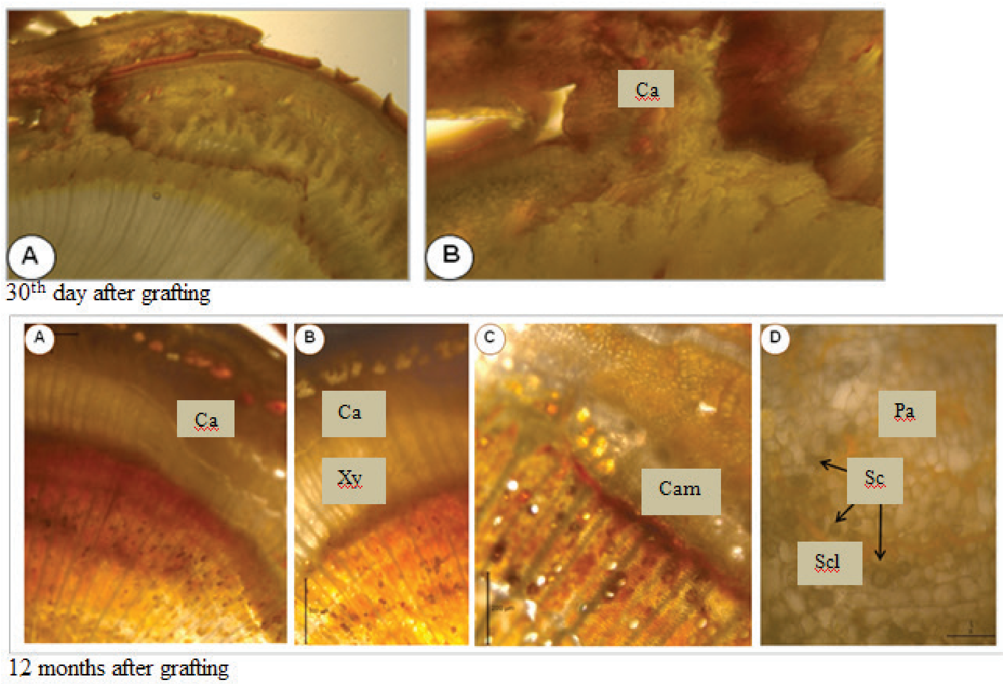


Figure 3- Cross sections taken 30 days and 12 months after the budding from budded parts of Laurenne cultivar on Rootpac R rootstock; A-B, callus cells (Ca), new xylem elements (Xy); C, cambium (Cam); D, parenchymatic cells (Pa), sclereids (Sc), sclerenchyma bundles (Scl.)

tissue properties and vascular differentiation increased. The differentiated cambium cells reached 6-8 rows (Figure 3).

It was observed that cambial continuity was established, vascular differentiation increased, the callus cells got regular parenchymatic tissue properties, and the parenchymatic cells were found to have a lot of sclereid cells in the cross sections taken from the budded parts on Rootpac 90 and Rootpac 70 rootstocks. In the samples budded onto Rootpac 70 rootstock, the differentiated cambium cells reached 6-8 rows (Figure 4, Figure 5).

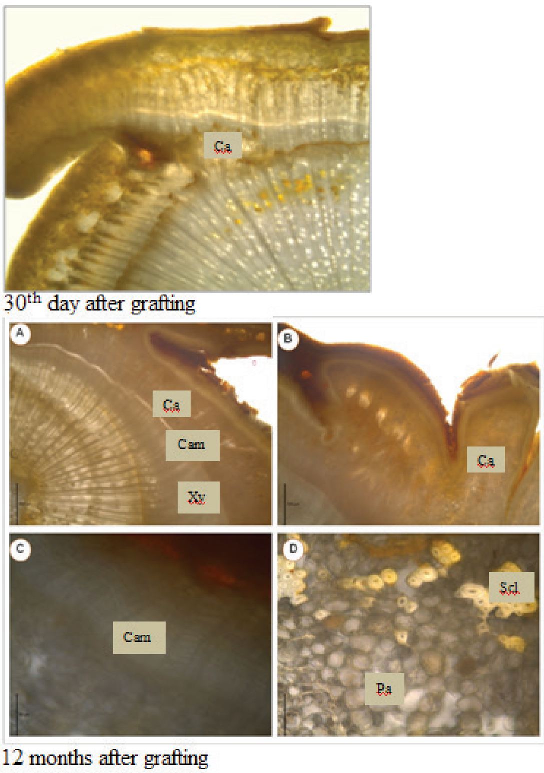


Figure 4- Cross sections taken 30 days and 12 months after the budding from budded parts of Laurenne cultivar on Rootpac 70 rootstock; A, callus cells (Ca), new xylem elements (Xy), cambium (Cam); B, callus cells (Ca); C, cambium (Cam); D, parenchymatic cells (Pa), sclerenchyma bundles (Scl.)

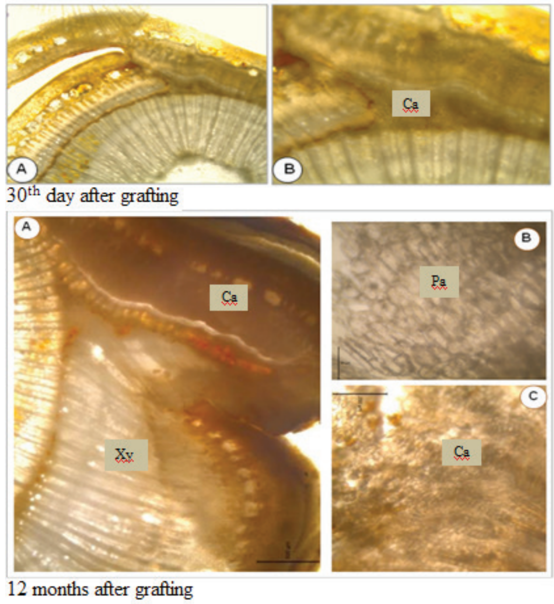
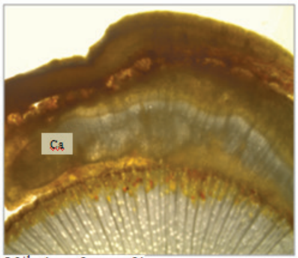


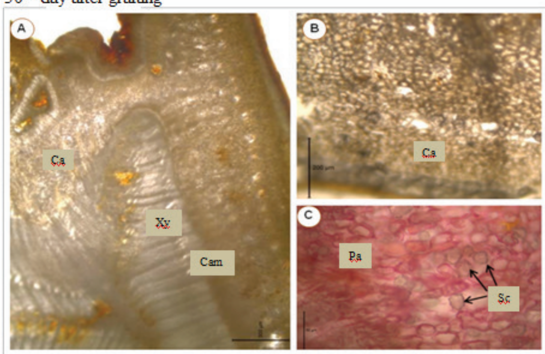
Figure 5- Cross sections taken 30 days and 12 months after the budding from budded parts of Laurenne cultivar on Rootpac 90 rootstock; A, new xylem elements (Xy), callus cells (Ca); B, parenchymatic cells (Pa); C, callus cells (Ca)

In the cross sections taken from the budded parts on Rootpac 40 rootstock, differentiation was similar to that of other rootstocks. Intensive callus formation was detected among the budded components, the differentiated cambium cells reached 5-8 rows, callus cells were found to gain regular parenchymatic tissue properties, and parenchymatic cells were found to contain a very large amount of sclereid cells (Figure 6).

Two plant parts, which are used as rootstock and scion, are required to be merged with each other over time and continue to live as a single plant in grafted combinations of fruit species. The connection between the rootstock and the scion takes place through the callus tissue, conflicting cambium tissues of rootstock and scion generate the callus formed by meristematic cells and these meristematic cells, which are composed of two different sources, merge along a line. The



30th day after grafting



12 months after grafting

Figure 6- Cross sections taken 30 days and 12 months after the budding from budded parts of Laurenne cultivar on Rootpac 40 rootstock; A-B, callus cells (Ca), new xylem elements (Xy), cambium (Ca); C, parenchymatic cells (Pa), sclereids (Sc)

formation of the callus, which begins to occur two days after the grafting, takes about 2-3 weeks, and the new cambium tissue forms from callus that fills between the rootstock and the scion. It is also indicated that completion of the vascular system has been occurred within 6-8 weeks. As a result of the union, both the rootstock and the scion side of the wood and phloem tissues allow the passage of water and plant nutrients with assimilation products from the point of graft union. The formation of callus tissue from both graft members, establishment of callus bridge, cambial differentiation, cambial continuity and completion phases of vascular system development; type of grafting, the method of grafting, grafting time can demonstrate alterations based on species used as rootstocks and scions. While the graft formation occurs in these phases at successfully developed compatible combinations, development disorders occur in cambial continuity and the development of vascular system phases at incompatible combinations. On the basis of these knowledges, following the status of the necrotic layers between the rootstock and the scion in the graftings, the formation of the callus tissue and establishment of the callus bridge, cambial differentiation and continuity also the formation status of new vascular tissues, it was reported that there may have been a reliable idea whether the combinations were compatible or incompatible (Hartmann & Kester 1961; Torabi 1975; Moore 1984; Tekintaş 1991; Hartmann et al 1997; Koyuncu et al 2007; Darikova et al 2011; Bayram et al 2014).

Grafting incompatibility between some new almond rootstocks and almond cultivars was anatomicaly examined in this study. It was determined that callus formation between the grafting components was completed within 30 days after grafting and new transmission bundles were occured after 12 months. Our findings are consistent with the results determined in anatomically investigated combinations of rootstock-scion relationships in different species. It was stated that the vascular differentiation between the graft components in the compatible combinations was seen within the next 2-3 weeks (15-20 days) after grafting (Mosse 1962; Hartmann et al 1990).

Ünal & Özçağıran (1986) established that cambium, xylem and phloem tissues occured with different quantities in each some pear/quince graft combination one month after the grafting and four months later, xylem and phloem tissues occured in places where cambium was regular. In a study conducted in hazelnut, the first cambial differentiation in callus tissue was determined in the sections taken after 18 days. Cambial continuity was determined to be established after 26 days of grafting on chip budding and 42 days after splice grafting (Balta 1993).

In graft combinations of *Prunus* species, it was reported that callus tissues occured one week after grafting, cambium cells occured after 10 days and vascular differentiation occured after 13 days (Errea & Felipe 1994; Errea et al 1994).

It was determined that formation of callus, cambium, new xylem and phloem was more rapid and regular in very good or good compatible peach/plum graft combinations while this formation was slow and deficient in incompatible or very poorly compatible combinations (Hartmann et al 1997), even the callus did not differ (Errea & Felipe 1994).

It was determined that callus cells in peach and nectarine cultivars grafted on almond rootstock occured within 14 days after grafting whereas vascular differentiation within 28 days. In the sections taken 4 months after the grafting, it was determined that the formation of cambium was completed in spite of locally interrupt, vascular differentiation continued but not completely in any combination (Tekintaş & Dolgun 1996).

Grafted samples which were examined 40 days after in walnut, the newly formed cambium tissue produced new vascular tissues (Kazankaya 1996). In some apricot cultivars grafted on Pixy rootstock, a weak callus formation occurred at the graft unions 15 days after grafting and cambial differentiation and new vascular tissues were identified in highly compatible cultivars (Kankaya et al 2001).

It was also detected that cambial continuity between the graft elements was achieved in all graft types within approximately 3 weeks after grafting at different buddings on *Pistacia vera* seedlings and this persistence was earlier in the T-budding than in the chip budding (Okay & Büyükkartal 2001). In the compatible combination of peach/plum, callus, cambium formation and vascular differentiation occured within four months after grafting, while a significant part of callus cells did not differentiate, cambium occured partly in some regions within a month after grafting, within 4 months after grafting, vascular differentiation didn't occur completely and necrotic layers were observed to increase in incompatible combinations (Demirsoy & Bilgener 2002; 2006).

Koyuncu et al (2007) stated that callus tissue between the rootstock and scion occured 2 weeks after grafting in the nectarine and peach cultivars that was highly compatible with the plum rootstock and the callus bridge was established also cambial continuity was achieved in following periods. Similar results were obtained in apricot cultivars grafted on some clonal rootstocks (Coşkun 2012).

An intense and regular callus formation occurred at early stage in all almond rootstocks used in our study similarly in all compatible combinations determined by other researches and no anatomical improvement was determined towards incompatibility in the investigated graft combinations.

4. Conclusions

It has been observed that callus cells were formed and started to develop in graft union taken after 30 days of budding, but cambial activity between rootstock and scion has not yet begun. This shows that in all rootstocks used, graft formation successfully continued at the graft union 30 days after budding. In all samples taken 12 months after budding, it was seen that graft formation in the budded parts has been completed successfully. Cambial continuity has been established, new transmission tissues have emerged, and callus cells have gained a regular parenchymatic tissue property. No significant difference was observed among rootstocks in terms of the period of callus cell formation after budding and establishment of cambial continuity. The Rootpac R, Rootpac 90 and almond seedling (high vigor) with Rootpac 70 and Rootpac 40 (medium vigor) showed almost the same process of differentiation. This indicates that Rootpac R, Rootpac 90, Rootpac 70 and Rootpac 40 clone rootstocks were successful in terms of graft compatibility with the Laurenne cultivar, as well as almond seedling. As a matter of fact, similar results were obtained in some studies in which the performance of different almond cultivars grafted on these rootstocks were evaluated (Pinochet 2010; Rubio-Cabetas 2015).

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