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Araştırma Makalesi/*Research Article (Original Paper)*

The Graft Unions of Almond, Plum and Apricot Varieties Grafted on Rootpac Rootstocks

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Abstract: The aim of this study was to obtain a basic information about the graft union formation in relation with growth characteristics between 'Rootpac' rootstocks and almond, plum and apricot varieties. In this trial, 'Rootpac-R', 'Rootpac-90', 'Rootpac-70', 'Rootpac-40', 'Rootpac-20' rootstocks and 'Nonpareil', 'Ferragnes' almond, 'Aprikoz', 'Hacıhaliloğlu' apricot and 'Black Diamond', 'Papaz' plum cultivars were used as plant materials. 1, 4 and 12 months after T budding, graft samples were evaluated microscopically and macroscopically. Transverse sections (20-45 µ) were cut by rotary microtome. At transverse sections that taken from 4 and 12 months after grafting were observed forming of callus in all combinations from scion and rootstocks, being broken of necrotic layer as different densities and occurring of cambial differentiations and cambial continuity between scion and rootstock were established. However, tissue development differences were observed in the combinations of 'Aprikoz' and 'Hacıhaliloğlu' apricots grafted on 'Rootpac-R', 'Black Diamond' plum grafted on 'Rootpac-40', and 'Black Diamond' plum, 'Papaz' plum, 'Aprikoz' and 'Hacıhaliloğlu' apricots grafted on 'Rootpac-20'.

Keywords: Cambial differentiation, Graft union, Rootstock, Scion

Rootpac Anaçlarına Aşılı Badem, Erik ve Kayısı Çeşitlerinin Aşı Kaynaşma Durumları

Öz: Bu çalışma, Rootpac anaçlarının badem, erik ve kayısı çeşitleri ile aşı kaynaşma durumlarını hakkında kısa sürede temel bir bilgi edinebilmek amacı ile yapılmıştır. Bu araştırmada bitki materyali olarak 'Rootpac-R', 'Rootpac-90', 'Rootpac-70', 'Rootpac-40', 'Rootpac-20' anaçları ve 'Nonpareil', 'Ferragnes' badem, 'Aprikoz', 'Hacıhaliloğlu' kayısı ve 'Black Diamond' ve 'Papaz' erik çeşitleri kullanılmıştır. T göz aşısı yöntemi ile oluşturulan kombinasyonlardan alınan 1, 4 ve 12 ay sonra aşı örnekleri mikroskobik ve makroskopik olarak değerlendirilmiştir. Enine kesitler (20-45 µ) rotary mikrotom ile kesilmiş ve enine kesitlerde aşılamadan 4 ve 12 ay sonra alınan, kalem ve anaçtan elde edilen tüm kombinasyonlarda kallus oluştuğu, farklı yoğunluklardaki nekrotik tabakaların kırıldığı ve kalem ile anaç arasında kambiyal farklılığın ve kambiyal sürekliliğin sağlandığı gözlenmiştir. Bununla birlikte, 'Rootpac-R' üzerine aşılanmış 'Aprikoz' ve 'Hacıhalioğlu' kayısı çeşitlerinin, 'Rootpac-40' üzerine aşılanmış 'Black Diamond' çeşidinin, 'Rootpac-20' üzerine aşılanmış 'Papaz' erik, 'Aprikoz' ve 'Hacıhaliloğlu' kayısı çeşitlerinin oluşturduğu kombinasyonlarda doku gelişiminde farklılıklar gözlenmiştir.

Anahtar kelimeler: Kambiyal farklılaşma, Aşı birleşme, Anaç, Kalem

Introduction

Rootstock is the most important material in the propagation of fruit trees. Thanks to the rootstocks, a fruit species or variety can be cultivated in soil and climatic conditions which are not suitable for it. However, rootstocks have an impact on the growth vigor, life, early or late cropping, productivity, fruit quality and ecological conditions and resistance against diseases and pests of trees. (Özçağıran 1974; Westwood 1995; Ağaoğlu et al. 1997; Zeynep et al. 2006). For this reason, it is targeted to reveal early cropping and highly productivity new rootstocks suitable for orchards where high density planting and re-planting are possible for more modern and profitable fruit growing in breeding studies. In line with these goals, 'Rootpac' series rootstocks were hybrid selected and developed by Agromillora Iberia, S. L., Barcelona, Spain. Pinochet (2010), studying the effect of the 'Big Top' variety on the 'Rootpac-R' rootstocks in Zaragoza, Spain, found that the cumulative yield and fruit weight increase relative to other rootstocks were high for chlorophyll content in leaves and tolerant to iron chlorosis. Furthermore, 'Rootpac-R' had showed good graft compatibility with Japanese plum and with numerous peach and nectarine cultivars such as 'Alexandra', 'Ambra', 'Britney Lane', 'Big Top', 'Catherine', 'Magique', 'May Crest', 'Romea', 'Subirana',

‘Summergrand’, ‘Sweet Dream’, and ‘Ufo-4’ when tested in nursery (3 years) and orchard trials (more than 4 years). It is also graft-compatible with the Spanish almond cultivars ‘Desmayo Largueta’ and ‘Moncayo’ as well as with ‘Nonpareil’, ‘Monterey’, and ‘Buttein California’. The clone had showed to be compatible with some apricot cultivars, although the range of tested cultivars is not extensive (Pinochet 2010). With the use of this new generation of rootstocks will be very important changes and developments in fruit growing. However, disagreement between some rootstocks and some varieties is a very important problem. There will be irreparable inconveniences for the establishment of the orchard without revealing suitable varieties/rootstocks combinations. For this reason, new clonal rootstocks should be used to control the compatibility of the cultivars to be grown before the orchard establishment. ‘Rootpac’ rootstocks, which have become widespread in the world and in Turkey, have also been required to carry out such a study in order to determine their compatibility with some almonds, apricots and plum varieties. ‘Nonpareil’, ‘Ferragnes’ from almond varieties, ‘Aprikoz’, ‘Hacıhaliloğlu’ from apricot varieties and ‘Black Diamond’, ‘Papaz’ from plum varieties are very important varieties especially for Turkey. If these varieties are determined to be compatible with the Rootpac series clonal rootstocks, Turkey will have significant distances in almond, apricot and plum cultivation. If orchard establishment is used with compatible varieties/rootstocks combinations, all the input costs will be reduced and the production costs will be reduced.

The aim of this study was to obtain a basic knowledge about the graft union formation in relation growth characteristics between ‘Rootpac’ rootstocks (‘Rootpac-R’, ‘Rootpac-90’, ‘Rootpac-70’, ‘Rootpac-40’, ‘Rootpac-20’) and almond (‘Nonpareil’ and ‘Ferragnes’), plum (‘Black Diamond’ and ‘Papaz’) and apricot (‘Aprikoz’ and ‘Hachaliloğlu’) cultivars.

Materials and Methods

This study was carried out in the Fruit Research Institute Egirdir-Isparta, Turkey. All cultivars and Rootpac series rootstocks were grafted by T bud method 4 September 2013. ‘Rootpac-R’, ‘Rootpac-90’, ‘Rootpac-70’, ‘Rootpac-40’ and ‘Rootpac-20’ clonal rootstocks and ‘Nonpareil’, ‘Ferragnes’ almond, ‘Aprikoz’, ‘Hacıhaliloğlu’ apricot and ‘Black Diamond’, ‘Papaz’ plum cultivars were used as plant materials. ‘Rootpac’ series (‘Rootpac-R’, ‘Rootpac-90’, ‘Rootpac-70’, ‘Rootpac-40’ and ‘Rootpac-20’) 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 2010). 45 plants were grafted for each combination. For histologic investigations graft samples were taken as 1 month and 4 months after from grafting. Samples of 3-5 graft unions from each rootstock/scion combinations were fixed in a fixative (FAA; 5ml formaldehyde + 5ml acetic acid + 90ml 70% ethyl alcohol) until the anatomical studies. Samples were cut with a Leica RM 2125 RT rotary microtome with thickness of 25-40µ. Sections of graft regions were stained with safranin and photographed (Olympus microscope). On these photographs; five developmental stages were examined during graft union formation; development and positions of necrotic layers, callus cells, formation of callus bridge at the graft interface, cambial continuity and formation of vascular tissues (Figure 1).

For macroscopic investigations graft samples were taken 12 months after from grafting. In longitudinal sections taken from the grafted site, starch accumulation was observed by examining the staining of rootstock and scion tissues with 1% iodized potassium iodide solution.

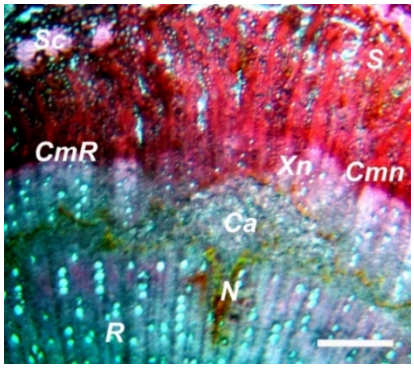

Figure 1. View of the side contactand graft surface with 25 microns in cross section taken 1 month after budding (Ca Callus, N necrotic area, CmR rootstock cambium, Sc Sclerenchyma tissues, Xn new xylem, Cmn new cambium, R rootstock).

Results

In general, cross sections of the graft union were examined 1 month after grafting and it was seen that new cambium tissues originated from callus tissues, and new xylem tissues were formed from these cambium tissues. Callus tissue

is formed in the gaps that emerge on both sides after removing the rootstock bark during grafting and between rootstock xylem and scion. In this tissue, necrotic layers and lines are encountered locally in amounts that vary by graft combination. These necrotic layers are sometimes adjacent to the xylem of rootstock and sometimes distributed in the callus tissue. Callus tissue formation is seen mostly in side gaps known as side pockets and in upper and lower junctions. The connection between rootstock and scion cortex tissues was obtained by means of the callus cells consisting of both. However, in the junction of 'Aprikoz'/'Rootpac-R', 'Hacıhaliloğlu'/'Rootpac-R', 'Black Diamond'/'Rootpac-40', 'Black Diamond'/'Rootpac-20', 'Papaz'/'Rootpac-20', 'Aprikoz'/'Rootpac-20', 'Hacıhaliloğlu'/'Rootpac-20' combinations, there were also some necrotic layers which caused discontinuities between tissues and the amount of which differed from graft to graft (Figure2). 4 months after grafting, callus tissues of the sections taken from the graft union generally maintained the condition they had 1 month after grafting. There were again necrotic layers and lines in the callus tissue formed in side pockets. But their amount varied by graft combination. The callus tissue in side pockets generally maintained its shape– i.e., protrusion towards the bark- in most of the graft combinations. Compared to those in the sections taken 1 month after grafting, both the callus tissue formed and the necrotic layers in it were less in amount, varying by graft combination. Nevertheless, in 'Aprikoz'/'Rootpac-R', 'Hacıhaliloğlu'/'Rootpac-R', 'Black Diamond'/'Rootpac-40', 'Black Diamond'/'Rootpac-20', 'Papaz'/'Rootpac-20', 'Aprikoz'/'Rootpac-20', 'Hacıhaliloğlu'/'Rootpac-20' combinations, the amount of callus tissues is increased compared to that of the callus tissues 1 month after grafting, causing local discontinuities in the newly formed xylem tissue. The discontinuity formed by necrotic layers occurred only at the junction of rootstock and scion cambiums (Figure 3).

In terms of development in the graft union, a strong shoot development was observed 12 months after grafting. In spite of a slight thickening of bark on the graft union, there was no thickening in the woody tissue. Rootstock and scion tissues were stained almost in the same intensity with IKI. However, tissue development differences were observed in the combinations of 'Aprikoz' and 'Hacıhaliloğlu' apricots grafted on 'Rootpac-R', 'Black Diamond' plum grafted on 'Rootpac-40', and 'Black Diamond' plum, 'Papaz' plum, 'Aprikoz' and 'Hacıhaliloğlu' apricots grafted on 'Rootpac-20' (Figure4).

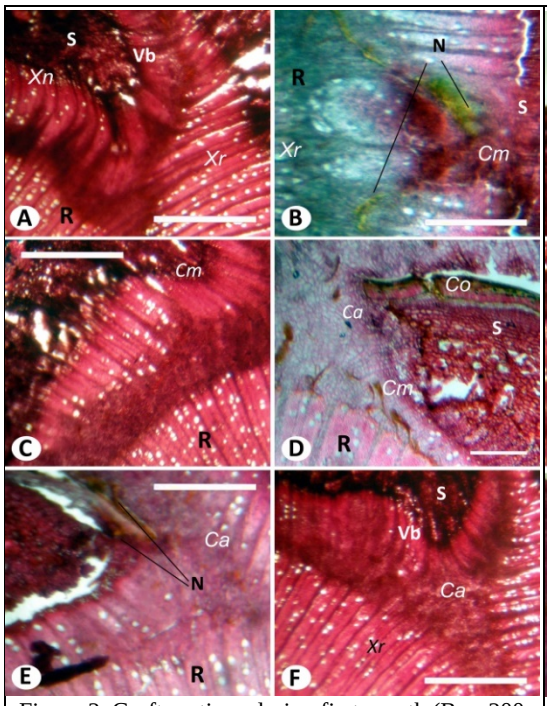

Figure 2. Graft sections during first month (Bar=200 µm) A-Papaz/Rootpac-20; B-Hacıhalioğlu/Rootpac-R; C-Hacıhalioğlu/Rootpac-20; D-Black Diamond/Rootpac-40; E-Aprikoz/Rootpac-R; F-Aprikoz/Rootpac-20 (S scion, R rootstock, N necrotic area, Xr rootstock xylem, Xn new xylem, Cm cambium, Ca callus, Co cortex, Vb vascular bond).

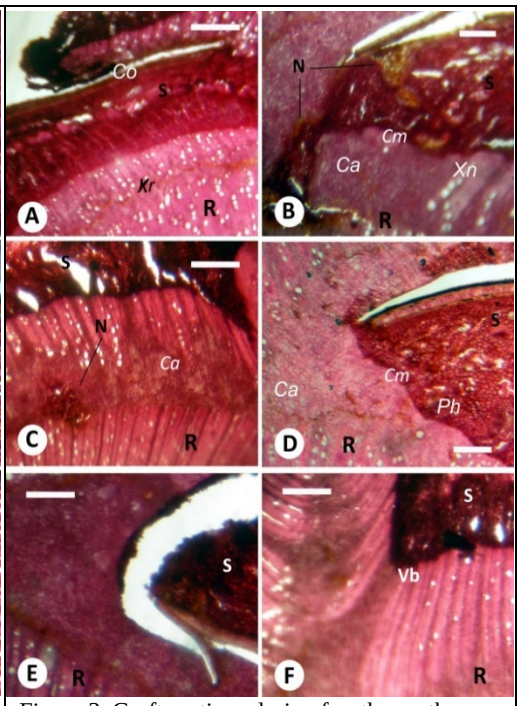

Figure 3. Graft sections during fourth month (Bar=100 µm) A-Papaz/Rootpac-20; B-Hacıhalioğlu/Rootpac-R; C-Hacıhalioğlu/Rootpac-20; D-Black Diamond/Rootpac-40; E-Aprikoz/Rootpac-R;F-Aprikoz/Rootpac-20. (S scion, R rootstock, N necrotic area, Xr rootstock xylem, Xn new ksilem, Cm cambium, Ca callus, Co cortex, Ph phloem, Vb vascular bond).

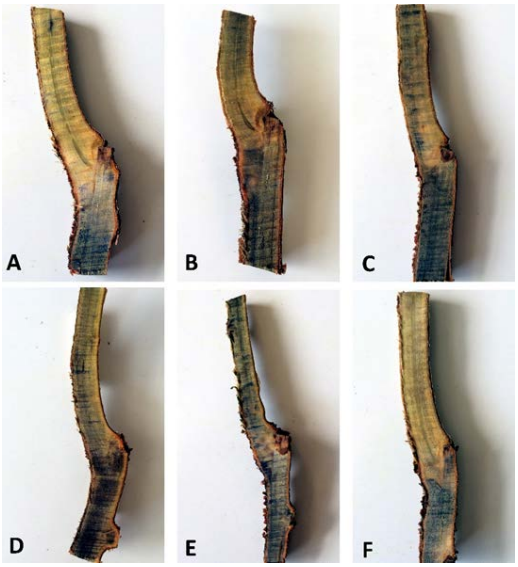

Figure 4. Graft region starch accumulation in twelfth month A-Papaz/Rootpac-20; B-Hacıhalioğlu/Rootpac-R; C-Hacıhalioğlu/Rootpac-20; D-Black Diamond/Rootpac-40; E-Black Diamond/Rootpac-20; F-Aprikoz/Rootpac-20.

Discussions

There are some important steps in graft formation for the success of a cultivar/rootstock combination. The first step includes establishing an intimate contact between rootstock and scion, matching the cambium tissues, and the beginning of callus formation (Errea et al. 1994). Callus tissues are produced separately in both of the graft components and they combine to enable the contact of rootstock and scion (Moore 1984). Callus tissues are formed in rootstock from young xylem cells, while they are formed from undamaged bark cambium, young phloem ray cells and sometimes from cortex in scion (Simons 1987). In order for callus cells to establish a callus bridge, they should make a connection between graft components by cracking the dead cell groups, which are injured and dead during grafting and turn brown because of the oxidation of phenolic components (Mosse 1962; Tekintaş 1991). Especially the existence of more intensive necrotic layers formed from scion creates significant problems for scion in the formation of callus bridge. However, it is known that necrotic layers easily break in callus cells in almost all successful graft combinations and do not pose too much of a problem as long as injuries during grafting are not excessive (Tekintaş 1991). In this research, in the cross sections taken after 1 month, callus formations, intensity of necrotic layers and breaking were generally within satisfactory limits. But, even so, these necrotic layers were more intensive and discontinuities were more noticeable in ‘Aprikoz’/ ‘Rootpac-R’, ‘Hacıhaliloğlu’/‘Rootpac-R’, ‘Black Diamond’/‘Rootpac-40’, ‘Black Diamond’/‘Rootpac-20’, ‘Papaz’/‘Rootpac-20’, ‘Aprikoz’/‘Rootpac-20’, ‘Hacıhaliloğlu’/‘Rootpac-20’ combinations. In a study conducted for the purpose of determining the compatibility levels of different *Prunus* clone and seedling rootstocks with some apricot cultivars, it was observed in the graft samples taken 1 month after grafting that new cambium tissues were formed and that rootstock and scion cambiums were discontinued in general with some necrotic layers and undifferentiated tissues (Bas 1998). The other two important steps of budding formation are cambial differentiation formation and continuity in the callus tissue formed from scion and also new vascular tissue formation. Many researchers reported that, in the studies conducted on different plant species, cambial differentiation occurred 2-3 weeks later (Hartmann and Kester, 1983; Hartmann et al.1990; Errea et al. 1994; Tekintaş and Dolgun, 1996;). Cambial continuity is highly important in terms of graft compatibility. The formation and continuity of cambial differentiation is generally observed in cultivar/rootstock combinations. However, it was notable that, in the combinations of ‘Aprikoz’ and ‘Hacıhaliloğlu’ apricots grafted on ‘Rootpac-R’, ‘Black Diamond’ plum grafted on ‘Rootpac-40’, and ‘Black Diamond’, ‘Papaz’ plums, ‘Aprikoz’ and ‘Hacıhaliloğlu’ apricots grafted on ‘Rootpac-20’, necrotic layers detected in the graft union were intensive and were not absorbed in the tissue unlike other combinations. But some researchers mentioned tissue problems due to different rootstock and scion thicknesses, tannin content in the fruit varieties followed up in terms of graft combinations, and excessive injuries likely to happen during grafting (Ünal 1992; Kankaya et al. 1999).

The macroscopic examinations performed on the graft combinations in the research 12 months after grafting showed that cambium, xylem and phloem tissues had a good continuity in all combinations and there was no difference between rootstock and scion tissues in terms of staining with IKI plum. These findings are similar to those of other researchers (Ünal 1984; Errea et al. 1994; Bas 1998; Koçak and Pırlak 2011; Tekintaş and Dolgun 1996; Zarrouk et al. 2006). Nonetheless, some differences were observed between ‘Aprikoz’/‘Rootpac-R’, ‘Hacıhaliloğlu’/‘Rootpac-R’, ‘Black Diamond’/‘Rootpac-40’, ‘Black Diamond’/‘Rootpac-20’, ‘Papaz’/‘Rootpac-20’, ‘Aprikoz’/‘Rootpac-20’,

'Hacıhaliloğlu'/'Rootpac-20' combinations and other combinations in terms of the quality of tissue development. Although the results and observations obtained from this study are similar to the graft combinations studied by many other researchers (Hartman and Kester 1983; Ünal and Tanrisever 1986; Tekintas 1988; Errea et al. 1994; Seferoğlu 1995; Tekintaş and Dolgun 1996; Zarrouk et al. 2006; Koçak and Pırlak 2011), further studies should be made to see if there will be any biochemical differences for 'Aprikoz'/'Rootpac-R', 'Hacıhaliloğlu'/'Rootpac-R', 'Black Diamond'/'Rootpac-40', 'Black Diamond'/'Rootpac-20', 'Papaz'/'Rootpac-20', 'Aprikoz'/'Rootpac-20', 'Hacıhaliloğlu'/'Rootpac-20' combinations and field observations (rootstock and scion diameter measurements) should continue in the coming years as well.

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References

Ağaoğlu S, Çelik H, Çelik M, Fidan Y, Gülşen Y, Günay A, Halloran N, Kösal D, Yanmaz R (2001) Genel Bahçe Bitkileri. Ankara Univ. Ziraat Fak. Eğitim, Araştırma ve Geliştirme Vakfı Yayınları, Ankara.

Baş M (1998). Farklı *Prunus* Klon ve Çöğür Anaçlarının Bazı Kayısı Çeşitleriyle Uyuşma Düzeyi, Bitki Besin Maddeleri Alımı ve Büyümeye Etkileri Üzerinde Araştırmalar. Çukurova Univ., Fen Bilimleri Enstitüsü,Doktora tezi, Adana, pp. 201.

Errea P, Felipe A, Herrero M (1994) Graft establishment between compatible and incompatible *Prunus* spp. Journal of Experimental Botany 45: 393-401.

Hartmann H T, Kester D, Davies F T (1990) Plant Propagation Principles and Practices. Fifth Edition. Regents/Prentice Hall, Englewood Cliffs, New Jersey.

Hartmann H T, Kester D E (1983) Plant Propagation Principles and Practices. Forth Edition, Printice-Hall, Inc., New Jersey, pp.727.

Kankaya A, Özyiğit S, Tekintaş F E, Seferoğlu G (1999) Bazı erik ve kayısı çeşitlerinin Pixy anacı ile uyuşmalarının belirlenmesi, Türkiye III. Ulusal Bahçe Bitkileri Kongresi, Ankara 1, 295–299.

Koçal H, Pırlak L (2011) Bazı *Prunus* ve klon ve çöğür anaçlarının Alyanak ve Roksana kayısı çeşitleriyle aşı uyuşma düzeylerinin belirlenmesi. Ege Üniversitesi Ziraat Fakultesi Dergisi 48(1): 39-45.

Moore, R., (1984). A model for graft compatibility-incompatibility in higher plants. American Journal of Botany 71(5): 752-758.

Mosse B (1962) Graft-incompatibility in fruit trees. Tech. Commun., No.28. Comm.Bur.Hort. and Plant Crops, East Malling, England.

Özçağıran R (1974) Meyve agaçlarında anaç ile kalem arasındaki fizyolojik ilişkiler. ege üniv. ziraat fak. meyve-bağ yetistirme ve islahı kursusu. Ege Üniv. Zir. Fak. Yayınları No. 243, Bornova. Izmir.

Pinochet J (2010) 'Replantpac' (Rootpac-R), a Plum–almond hybrid rootstock for replant situations. HortScience 45(2): 299–301.

Seferoğlu H G (1995) Compatibility of some plum varieties with apricot rootstock. Ten International Symposium on apricot culture 384: 505-509.

Simons R K (1987) Compatibility and stock-scion interactions as related to dwarfing (Eds. Rom C.R. and Carlson, F.R.). Rootstocks for Fruit Crops. John Wiley Sons., New York, USA, 79-106.

Tekintas F E (1988) Cevizlerde (*J. regia*) Aşı kaynaşması ve aşı ile ilgili sorunlar üzerine araştırmalar. Ege Univ., Fen Bilimleri Enstitüsü, Doktora tezi, İzmir, pp. 105.

Tekintaş F E (1991) Farklı anaçlar üzerine aşılanan turunçgil tür ve çeşitlerinde kaynaşmanın anatomik ve histolojik olarak incelenmesi üzerinde araştırmalar. YüzüncüYıl Univ. Ziraat Fakültesi Dergisi 21(3): 399-407.

Tekintas F E and Dolgun O (1996) An investigation on compatibility in some peach and nectarin cultivars grafted on almond seedlings, YüzüncüYıl Univ. Ziraat Fakültesi Dergisi 6(1): 51-54.

Ünal A (1984) Ayva anaçlarının bazı armut çesitleriyle uyuşma durumları üzerinde arastırmalar. Ege Univ. Ziraat Fakültesi Dergisi 20(3): 141-155.

Ünal A and Tanrısever A (1986) Bazı ayva ve armut çesitlerinde kalburlu boruların yapıları ve bunların uyuşmazlıkla ilişkileri üzerine arastırmalar. Doga Tarım Orman Dergisi 10(2): 288-297.

Ünal A (1992) Seftali cogur anaclarinin bazi badem, kayısı, erik çeşitleriyle oluşturduğu aşı kombinasyonlarında aşıyerinin anatomik yapısının özellikleri ile ilgili araştırmalar. Türkiye I. Ulusal Bahçe Bitkileri Kongresi, Izmir, 1: 41-45.

Westwood N M (1995) Temperate Zone Pomology, Plysiology and Culture. Timber press. Portland. Oregon.

Zarrouk O, Gogorcena Y, Moreno M A, Pinochet J (2006) Graft compatibility between peach cultivars and *Prunus* rootstocks. Hortscience 41(6): 1389-1394.

Eroğlu Ö Z, Mısırlı A, Küden A (2016) The cross-breeding performances of some peach varieties. Yüzüncü Yıl Üniv. Tarım Bilimleri Dergisi 26(1): 89-97.