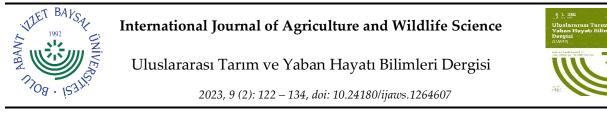
# PAPER DETAILS

TITLE: Anatomical and Histological Development of the Union of Splice Grafting in Hazelnut

AUTHORS: Fikri BALTA

PAGES: 122-134

ORIGINAL PDF URL: https://dergipark.org.tr/tr/download/article-file/3008604



## Anatomical and Histological Development of the Union of Splice Grafting in Hazelnut\*

Fındıkta Dilciksiz Aşı Tekniğinde Kaynaşmanın Anatomik ve Histolojik Gelişimi

Fikri Balta<sup>1</sup>

Received: 13.03.2023

Accepted: 15.06.2023 Published: 21.08.2023

Abstract: The anatomical and histological development of the graft union in splice grafting in hazelnut (*Corylus avellana* L.) was examined. One-year suckers were used as rootstock and scion materials. Grafting was done manually in the first week of December. The grafted plants were kept for 28 days at 26-28 °C and 70-80% relative humidity conditions. The cross and longitudinal sections with a thickness of 12-20 microns taken from the graft union area by a rotary microtome 14, 18, 21, 26, 32, 52 ve 140 days after grafting were examined microscopically. Early callus proliferation from rootstock and particularly scion two weeks after grafting was generally slow. The initial cambial differentiations in callus tissues were seen in sections 18 days after grafting. Cambial continuity between rootstock and scion was established 32 days after grafting. The sections in the following periods exhibited that the graft partners were in vascular relationship. The development of the graft union was successfull with all its stages. It was observed that the amount of callus tissue proliferated during the first two weeks after grafting directed the subsequent development of the union. In this respect, it is thought that developing methods that encourage callus formation in the early periods of the union will increase the success in related studies. The anatomical and histological examinations revealed that rootstock and scion thicknesses that are very close to each other and accordingly well matching of graft partners from cambial zones affect the development of union positively and prepare a suitable basis for early vascular differentiation. **Keywords:** Hazelnut, *Corylus avellana* L., Splice grafting, Graft union

&

Öz: Çalışmada fındıkta (*Corylus avellana* L.) dilciksiz aşılama tekniğinde aşı kaynaşmasının anatomik ve histolojik gelişimi incelenmiştir. Anaç ve kalem materyali olarak bir yıllık dip sürgünleri kullanılmıştır. Aralık ayının ilk haftasında yapılan aşılar 26-28 C° sıcaklık ve % 70-80 nisbi nem koşullarında 28 gün tutulmuştur. Aşılamadan 14, 18, 21, 26, 32, 52 ve 140 gün sonra aşı yerlerinden rotary mikrotomla 12-20 mikron kalınlığında alınan kesitler fotomikroskop altında incelenmiştir. Aşılamadan iki hafta sonraki kesitlerde anaç ve özellikle kalemden kallus oluşumunun yavaş gerçekleştiği görülmüştür. Kallus dokusunda ilk kambiyal farklılaşmalara aşılamadan 18 gün sonraki kesitlerde rastlanmıştır. Aşı elemanları arasında kambiyal devamlılık aşılamadan 32 gün sonra kurulmuştur. İlerleyen dönemlerdeki kesitlerde aşı parçalarının vasküler ilişki içerisinde oldukları belirlenmiştir. Kaynaşmanın gelişimi tüm aşamalarıyla başarıyla gerçekleşmiştir. Aşılama sonrası ilk iki haftalık dönemde oluşan kallus miktarının kaynaşmanın sonraki gelişimine yön verdiği belirlenmiştir. Bu bakımdan, ilgili araştırmalarda kaynaşmanın erken dönemlerinde kallus oluşumunu teşvik edici yöntemlerin geliştirilmesinin başarıyı yükselteceği düşünülmektedir. Anatomik ve histolojik incelemeler, birbirine çok yakın anaç ve kalem kalınlığının ve buna bağlı olarak aşı parçalarının kambiyal bölgelerden iyi çakıştırılmasının kaynaşmanın seyrini olumlu etkilediğini ve erken vaskuler farklılaşmalara zemin hazırladığını ortaya koymuştur.

Anahtar Kelimeler: Fındık, Corylus avellana L., Dilciksiz aşı, Aşı kaynaşması

Cite as: Balta, F. (2023). Anatomical and histological development of the union of splice grafting in hazelnut. International Journal of Agriculture and Wildlife Science. 9 (2), 122-134. doi: 10.24180/ ijaws.1264607

Plagiarism/Ethic: This article has been reviewed by at least two referees and it has been confirmed that it is plagiarism-free and complies with research and publication ethics. https://dergipark.org.tr/tr/pub/ijaws

Copyright © Published by Bolu Abant Izzet Baysal University, Since 2015 – Bolu

<sup>&</sup>lt;sup>1</sup> Prof. Dr. Fikri Balta, Ordu University, Department of Horticulture, baltaf04@yahoo.com

<sup>\*</sup> This study is produced from a part of the PhD thesis of the author.

#### INTRODUCTION

Grafting provides many advantages such as benefiting from the characteristics of various rootstocks, changing varieties, encouraging early fruiting, increasing productivity, obtaining quality fruit, and large-scale propagation of fruit species that cannot be serially propagated by other vegetative methods (Hartmann and Kester, 1974; Goldschmidt, 2014; Adhikari et al., 2022). Therefore, it has an important place in horticulture (Melnyk, 2016).

Grafting is a plant's response to injury related to its ability to regenerate (Moore, 1981; Melnyk, 2016). This response varies over time during the year under uncontrolled conditions (Adhikari et al., 2022). In grafting, after the cut parts of the rootstock and scion contact each other (adhesion), cell divisions occur and then the vascular tissues differentiate and the two plant parts turn into a single plant (Melnyk & Meyerowitz, 2015).

In grafting, well matching of rootstock and scion and the close thickness of the graft partners affect the success and development of the union positively (Tekintaş, 1988). This detail is especially important for nut species such as hazelnuts, which have been reported to have difficulties in propagation by grafting (Lagerstedt, 1981). At the graft union, callus formation from rootstock and scion, establishment of the callus bridge at the graft interfaces, cambial differentiation, cambial continuity, establishment of the vascular system can be affected by species, cultivar, rootstock, grafting technique, grafting time, and environmental conditions during and after grafting (Farsi et al., 2016).

During the graft union, anatomical, physiological, biochemical and molecular changes occur in the plant that may affect important horticultural characteristics (Goldschmidt, 2014; Habibi et al., 2022). Knowing the anatomical and histological development of the union in grafting studies can offer some important insights not only for the formation of successful grafting combinations and the selection of the grafting technique (Tekintaş, 1988; Farsi et al., 2016), but also for transferring the grafted plant to the appropriate conditions at the right time. Indeed, early establishment of the union in grapevine grafts reduced posttransplant losses of grafted plants (Cangi et al., 2000). Various anatomical and histological studies on the graft union have been reported in many fruit species (Ünal and Özçağran, 1986; Asante and Barnett, 1997; Tekintaş, 1988; Watanabe & Nakazatomi, 1990; Polat and Kaşka, 1992; Seferoğlu et al., 2004; Serdar et al., 2005; Demirsoy and Bilginer, 2006; Dolgun et al., 2008; Polat et al., 2010; Kalkışım and Tekintaş, 2011; Farsi et al., 2016; Özdemir et al., 2019).

Today's modern hazelnut culture foresees the cultivation of hazelnut plant in the form of a single-trunk tree suitable for mechanization (Bijelić et al., 2021). In this respect, although the efforts on hazelnut grafting have gained importance, studies on the subject in our country are very limited. In this study, it was aimed to examine the anatomical and histological aspects of the development of the graft union in splice grafting technique depending on time in hazelnut, and the issues that may affect the development of union were emphasized.

## MATERIAL AND METHOD

The research was carried out at Yüzüncü Yıl University, Faculty of Agriculture, Department of Horticulture. As rootstock and scion material, one-year suckers (*Corylus avellana* L.) obtained from hazelnut grower's orchards in Karaca neighborhood of Çarşamba district of Samsun were used. One-year suckers were brought from Samsun to Van in mid-November in sacks containing moist sawdust. They were kept at room conditions until grafting. The scions were prepared with 2-3 buds from suckers. Grafting was done manually on one-year suckers using the splice technique (Figure 1) in the first week of December. The grafted plants were kept under controlled conditions at 26-28 °C and 70-80% relative humidity for 28 days. They were transferred to a room with a temperature of 16-20 °C and a relative humidity of 80-90% and surrounded by PVC plastic. When the outside weather conditions are suitable, they were moved to the greenhouse and left to develop.

123



**Figure 1**. Splice grafting of hazelnut. *Şekil 1. Dilciksiz fındık aşısı.* 

# Preparation of graft site samples

In order to examine the anatomical and histological development of the union, the graft sites of the grafted plants were cut by grafting scissor 14, 18, 21, 26, 32, 52 and 140 days after grafting and kept in 70% ethanol until paraffin method. As a preliminary preparation for the paraffin method, the parts of the graft sites were cut into small pieces with the help of a razor blade.

These small pieces were treated with a series of solutions (Table 1). Hard paraffin, equal to 1/3 of the amount of xylol, was added to the containers in which the samples were contained, and they were kept in unheated room conditions for 1 day. Then, small pieces of the graft sites were saturated with paraffin in an oven at 62-63 °C for 2-3 months.

Order	Treatment	Duration (hour)
1	80% ethyl alcohol	6
2	90% ethyl alcohol	16
3	Pure alcohol	1
4	3 volumes of pure alcohol + 1 volume of xylol	2
5	2 volumes of pure alcohol + 2 volume of xylol	2
6	1 volumes of pure alcohol + 3 volume of xylol	2
7	Xylol	2

**Table 1.** Treatments for small pieces of graft site samples.

 *Cizelge 1. Ası veri örnek parcalarına uygulanan islemler.*

# Sectioning and Staining

Paraffin-saturated pieces were carefully embedded in blocks for sectioning. These blocks were then placed in the rotary microtome. Cross and longitudinal sections with a thickness of 12-20 microns were taken with a rotary microtome and permanent preparations were prepared. Sections were stained in three ways: safranin/fast green double staining, safranin and fast green. In the staining process, it was aimed to clearly distinguish the tissues in the sections under photomicroscope. Treatments for safranin/fast green double staining are given in Table 2. In staining with fast green, the treatments other than the 8th, 9th, 10th, 11th and 12th orders, and the treatments other than the 13th order in staining with safranin were followed (Table 2).

Table 2. Safranin/Fast green double staining.
Çizelge 2. Safranin/Fast Green ikili boyama işlem aşamaları.

Order	Treatments	Duration
1	3 volume of xylol + 1 volumes of pure alcohol	10 min.
2	2 volume of xylol + 2 volumes of pure alcohol	10 min.
3	1 volume of xylol + 3 volumes of pure alcohol	10 min.
4	Pure alcohol	1 min.
5	90% ethyl alcohol	4 min.
6	80% ethyl alcohol	4 min.
7	70% ethyl alcohol	4 min.
8	% 1 safranin	30-45 min.
9	Washing with tap water	1.5 min.
10	% 0.5 picric acid	1-2 min.
11	96% ethanol with a few drops of ammonia	1-2 min.
12	96% ethyl alcohol	10 sec.
13	% 1 fast green	10 sec.
14	1 volume of xylol + 1 volumes of pure alcohol	15-20 sec.
15	Pure alcohol with a few drops of xylol	2-3 sec.
16	Release in xylol followed by coating with Canadian	
	balsam	

## **Examination of Sections**

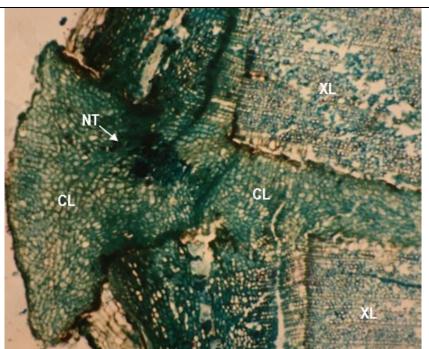
Cross and longitudinal sections taken from the graft unio sites were examined and photographed under a photomicroscope (Olympus BH-2). At the union sites, the state of callus tissues formed by rootstock and scion, the state of necrotic layers, the union of rootstock and scion, cambial differentiation, establishment of cambial continuity between rootstock and scion, and the formation of new vascular tissues on the sections were examined and evaluated.

## RESULTS

In the cross-sections 14 days after grafting, callus proliferation at the graft union site was satisfactory in some of the graft samples (Figure 2) and generally weak in others. Callus proliferation in the upper and lower parts of the union site was more than in the middle parts. Thin-membrane and high turgor callus cells were produced from cambium, young xylem and phloem tissues of rootstock and scion that were not destroyed during grafting. Although callus tissue extended uninterruptedly in the upper, lower and middle parts of the graft site, it has not fully expanded between the graft interfaces. Therefore, it was generally considered weak at this stage. It was observed that the necrotic layers were largely broken by the callus tissue. Initial cambial cell differentiations were not observed within the callus tissue at the graft union site.

Cross-sections of successful graft samples 18 days after grafting showed more callus proliferation. The callus formation has expanded towards the junction surfaces between the xylem tissues of the graft partners, but has not completely filled all graft interfaces. In successful graft samples where rootstock and scion thicknesses are very close to each other, the initial cambial differentiations and extensions were seen at the graft union site. The cambial differentiation, which is almost in line with the original cambium of the rootstock and scion within the callus tissue, expanded towards the inner parts and turned into a cambial extension (Figure 3). However, although the cambial extensions that developed separately from both the rootstock and the scion were in contact with the original cambium tissues of the graft partners, they could not yet unite with each other in the callus tissue formed towards the graft interfaces. Therefore, cambial continuity was not established between the graft partners in this period.





**Figure 2.** The appearance of the tissues in a cross-section of the union site 14 days after grafting. CL: Callus, NT: Necrotic layer, XL: Xylem (Safranin/Fast green, 4x10). *Şekil 2. Aşılamadan 14 gün sonra aşı bölgesinden alınan enine kesitte dokuların görünümü. CL: Kallus, NT: Nekrotik tabaka,* 

XL: Ksilem (Safranin/Fast Green, 4x10).

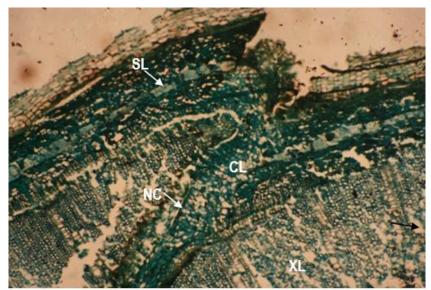


Figure 3. The appearance of the tissues in a cross-section of the union site 18 days after grafting. CL: Callus, NC: New cambium, SL: Sclerenchymatic fiber cells, XL: Xylem (Safranin/Fast green, 4x10).
Şekil 3. Aşılamadan 18 gün sonra aşı bölgesinden alınan enine kesitte dokuların görünümü. CL: Kallus, NC: Yeni Kambiyum, SL: Sklerankimatik lif hücreleri, XL: Ksilem (Safranin/Fast green, 4x10).

Cambial differentiations and extensions in the callus tissue continued progressively in the cross-sections 21 days after grafting. The new cambial extensions of the rootstock and scion, which connect with the original cambium of the graft partners, have not yet connected with each other in the callus tissue formed towards the inner surfaces. Therefore, cambial continuity between rootstock and scion has not yet been established in the graft samples of this period. However, while it was observed that new xylem tissues



were produced from new cambium tissues in places, sclerenchymatic cell groups were seen in the callus tissue (Figure 4). It was evaluated that the necrotic layers did not pose a problem in terms of the development of union.

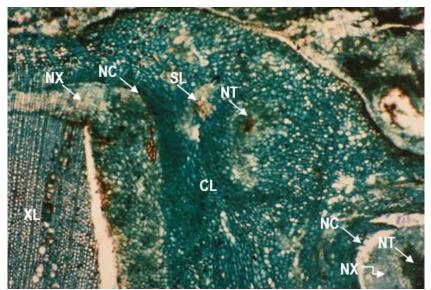


Figure 4. The appearance of the tissues in a cross-section of the union site 21 days after grafting. CL: Callus, NC: New Cambium, NX: New Xylem, NT: Necrotic layer, SL: Sclerenchymatic fiber cells (Safranin/Fast green, 4x10).
 Şekil 4. Aşılamadan 21 gün sonra aşı bölgesinden alınan enine kesitte dokuların görünümü. CL: Kallus, NC: Yeni Kambiyum, NX: Yeni Ksilem, NT: Nekrotik tabaka, SL: Sklerankimatik lif hücreleri, (Safranin/Fast green, 4x10).

Although callus formation at the graft site was satisfactory in cross-sections of successful graft samples 26 days after grafting, callus tissue did not completely fill all interfaces of the union in most of the graft samples. The cambial continuity was established between rootstock and scion. It was observed that cambial continuities were established as slightly or sharply curved in the graft partners. Differentiated new xylem elements were seen on the rootstock and scion sides (Figure 5). Callus cells formed more uniform shapes and rows at the union sites. Necrotic layers were generally well fragmented.

In cross-sections 32 days after grafting, not all cut surfaces between graft partners were fully filled with callus tissue. In successful graft samples, cambial continuity was established between graft partners (Fig. 6). In the longitudinal sections, the new vascular cambium tissue extended uninterruptedly within the callus tissue proliferated by rootstock and scion (Figure 7). In this period, after cambial continuity, it was observed that new vascular tissues were produced in places at the graft union site.

In the cross-sections 40 days (Figure 8) and 52 days (Figure 9) after grafting, vascular differentiations continued within the callus tissue, more new xylem elements were produced by especially rootstock. Callus cells were located in the union zone, adjacent to the newly formed vascular tissues and forming regular rows. In some graft samples, cambial continuity was curved towards the middle part of the union (Figure 9). Necrotic areas were seen in places adjacent to cambial differentiations. The establishment of cambial continuity was affected by these areas. Cambial continuity is shaped like a straight line in some samples, and curved in some samples by narrowing and expanding in places. Thus, the extension pattern of the new vascular cambium also affected the positions of the newly formed vascular tissues. Sclerenchymatic cell groups were encountered in the callus tissue (Figure 8).



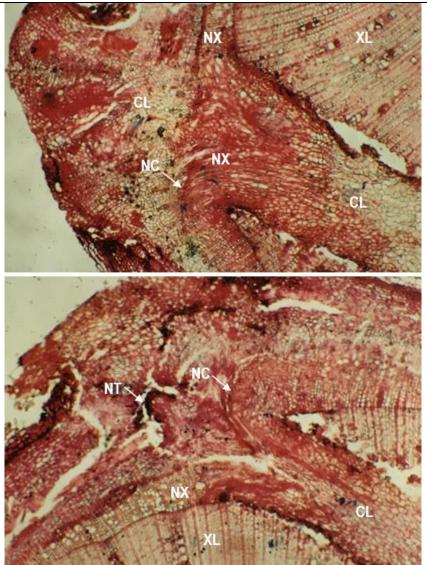
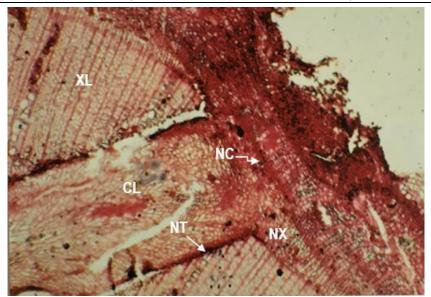


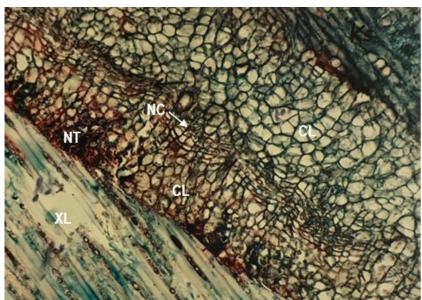
Figure 5. The appearance of the tissues in a cross-section of the union site 26 days after grafting. CL: Callus, NT: Necrotic layer, NC: New Cambium, NX: New Xylem, XL: Xylem (Safranin, 4x10).
Şekil 5. Aşılamadan 26 gün sonra aşı bölgesinden alınan enine kesitte dokuların görünümü. CL: Kallus, NT: Nekrotik tabaka, NC: Yeni Kambiyum, NX: Yeni Ksilem, XL: Ksilem (Safranin, 4x10).

128



**Figure 6.** The appearance of the tissues in a cross-section of the union site 32 days after grafting. CL: Callus, XL: Xylem, NT: Necrotic layer, NC: New Cambium, NX: New Xylem (Safranin, 4x10).

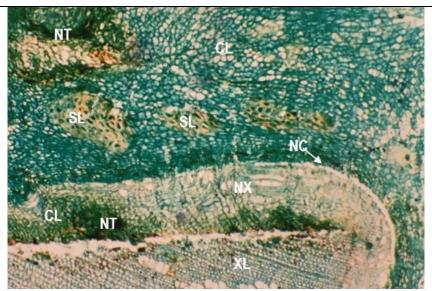
Şekil 6. Aşılamadan 32 gün sonra aşı bölgesinden alınan enine kesitte dokuların görünümü. CL: Kallus, NT: Nekrotik tabaka, NC: Yeni Kambiyum, NX: Yeni Ksilem, XL: Ksilem (Safranin, 4x10).



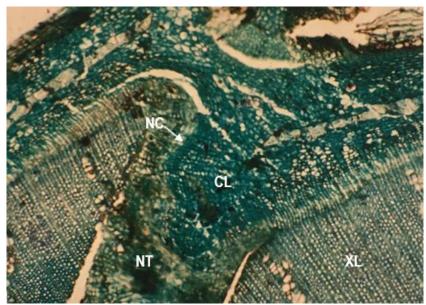
**Figure 7**. The appearance of the tissues in a longitudinal section of the union site 32 days after grafting. CL: Callus, NT: Necrotic layer, NC: New Cambium, XL: Xylem (Safranin/Fast green, 10x10).

Şekil 7. Aşılamadan 32 gün sonra aşı bölgesinden alınan boyuna kesitte dokuların görünümü. CL: Kallus, NC: Yeni Kambiyum, NT: Nekrotik tabaka, XL: Ksilem (Safranin/Fast green, 10x10).

130



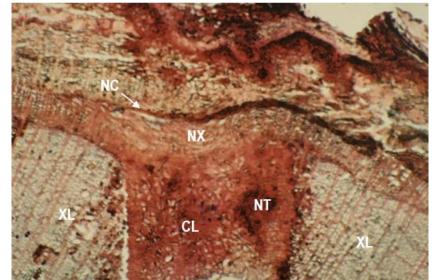
- Figure 8. Tissue differentiation within the callus in a cross-section of the union site 40 days after grafting. CL: Callus, NC: New Cambium, NT: Necrotic layer, SL: Sclerenchymatic fiber cells, NX: New Xylem, XL: Xylem (Fast green, 4x10).
  - Şekil 8. Aşılamadan 40 gün sonraki enine kesitte kallus içindeki doku farklılaşmaları. CL: Kallus, NC: Yeni Kambiyum, NT: Nekrotik tabaka, SL: Sklerankimatik lif hücreleri, NX: Yeni Ksilem, XL: Ksilem (Fast green, 4x10).

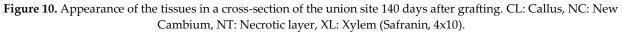


**Figure 9.** The appearance of the tissues in a cross-section of the union site 52 days after grafting. CL: Callus, NC: New Cambium, NT: Necrotic layer, XL: Xylem (Safranin/fast green, 4x10).

Şekil 9. Aşılamadan 52 gün sonra aşı bölgesinden alınan enine kesitte dokuların görünümü. CL: Kallus, NC: Yeni Kambiyum, NT: Nekrotik tabaka, XL: Ksilem (Safranin/Fast green, 4x10).

Anatomical and Histological Development of the Union of Splice Grafting in Hazelnut





Şekil 10. Aşılamadan 140 gün sonra aşı bölgesinden alınan enine kesitte dokuların görünümü. CL: Kallus, NC: Yeni Kambiyum, NT: Nekrotik tabaka, NX: Yeni Ksilem, XL: Ksilem (Safranin, 4x10).

140 days after grafting, it was observed in the cross-sections of the graft samples that shoot development continued, that the graft partners were in vascular relationship with each other (Figure 10) and the union progressed successfully. Callus cells forming regular rows are located in the union site as parenchymatic cells. Necrotic layers were present in different parts of the union as fragmented and absorbed. On the other hand, sections from unsuccessful graft samples were also examined, and the failing aspects of union were evaluated. The development of union was not interrupted in successful grafts where the rootstock-scion thickness was close to each other and well matched from the cambial zones. However, in unsuccessful grafts where the rootstock and scion did not match well, the graft partners could not establish cambial and vascular relationships with each other because their cambial regions were far from each other.

# DISCUSSION

The scientific knowledge about the mechanisms underlying graft union in plants is limited (Moore, 1984; Goldschmidt, 2014). As a result of the re-establishment of vascular connections between graft partners, long-distance signal transmission between tissues is ensured, cell-to-cell communication is largely mediated by plasmodesmata channels in the cell wall, which symplastically connect adjacent cells, and thus rootstock and scion cells form symplastic connections (Pina et al., 2012; Kurotani and Notaguchi, 2021; Amsbury, 2022).

Adhesion of graft partners, callus formation, establishment of callus bridge, cambial differentiations within the callus tissue, cambial continuity, production of new vascular tissues from the new cambium, and establishment of a functional vascular system at the graft site constitute the stages of the graft union (Hartmann and Kester, 1974; Goldschmidt, 2014). Grafting success and the graft union can be affected by many factors such as mechanical mismatches of rootstock and scion, desiccation of the tissues, adverse environmental conditions, failure of callus initiation, failure of adequate vascular differentiation, or physiological rejection between the tissues (Farsi ve ark., 2016).

In the first stage of graft union, callus tissue is mutually formed after the adhesion of rootstock and scion. Adequate callus formation at the graft union site in the early period and the establishment of callus bridge between the graft partners are necessary or the success of the graft. Because, in the later stages of union, the differentiation of the new cambium tissue and the establishment of cambial and vascular continuity between graft partners are realized through the callus tissue.



Callus tissue also plays a role in the disappearance of necrotic layers formed as a result of injury at the graft interfaces. In this study, callus formation at the graft interfaces was generally slow in the first 2 weeks after grafting. The callus tissue did not fill all the interfaces in many graft samples, even in the later stages of union. Necrotic layers were better disappeared in graft samples with sufficient callus. It has been determined that the amount of callus tissue formed in the early stages after grafting directed the development of union, and is effective on the disappearance of necrotic layers. Similar anatomical observations were reported by Şenyurt (2017), who examined the development of union in hazelnut cultivars grafted on *Corylus colurna* L. rootstock. It has been reported that callus formation occurs more slowly in hazelnuts during the graft union compared to fruit species such as apple and pear, and regular high temperatures are required for callus formation (Lagerstedt, 1981; Lagerstedt, 1984). Farsi et al. (2016) reported that intensive necrotic layers and weak callus formation at the graft site caused the failure and reduced the survival percentage of plants after transplanting. In this respect, it is thought that the development of methods that encourage callus formation in hazelnut grafting efforts will increase the grafting success.

The second stage of graft union is cambial differentiation within the callus tissue (Mahunu et al., 2012). In related studies, different findings have been reported on the initiation of the first cambial differentiations in the callus tissue after grafting. Farsi et al. (2016) reported that first cambial cell differentiation in callus tissue occurred 14 days after grafting in walnut grafts. Kurt and Tekintaş (2020) observed it 30 days after grafting in *Pinus pinea* L. grafts. It was observed 21 days after grafting in Erciş and Hafızali grape varieties grafted on 5BB rootstock (Cangi et al., 2000). In this study, the first cambial differentiations in callus tissue were seen in sections taken 18 days after grafting.

Establishment of cambial continuity following cambial differentiations is essential for the production of vascular tissues at the graft union site and ultimately for successful completion of union (Mahunu et al., 2012). The establishment time of cambial continuity on the callus bridge between rootstock and scion may vary according to graft combinations, grafting techniques and various applications. Establishment time of cambial continuity between graft partners after grafting were reported to be 3 weeks in pistachio grafts (Okay and Büyükkartal, 2001), 24 days in chip budding and 40 days for patch budding in walnut (Tekintaş, 1988), 25-35 days for omega grafts in walnut (Balta et al., 1996a), 45 days in citrus grafting combinations (Tekintaş, 1991), 45 days in chestnut grafts (Balta et al., 1993), 22-30 days for İskenderiye Misketi grape variety grafted on Rupestris du Lot rootstock (Balta et al., 1996b), 35-42 days Erciş and Hafızali grape varieties grafted on 5BB rootstock (Cangi et al., 2000), 30 days minigrafting of walnut (Farsi et al., 2016), 4 weeks in hazelnut patch budding on *Corylus colurna* L. seedling rootstocks (Şenyurt, 2017), 90 days in *Pinus pinea* L. grafts (Kurt and Tekintaş, 2020) and 100 days in loquat (*Eriobotrya japonica* L.) grafts (Polat and Kaşka, 1992). In this study, cambial continuity between graft partners was established 32 days after grafting. It took shape at the graft union site according to the matching position of rootstock and scion and the density of necrotic areas.

Establishment of vascular tissue connections in graft union constitutes the last stage (Mahunu et al., 2012). Post-grafting vascular tissue connection in higher plants is usually established 6 to 8 weeks after grafting (Moore, 1981). At the graft union site, new vascular tissues are produced by the new cambium tissue. In this study, graft partners were in vascular connection and relationship in sections taken 52 days after grafting and in the following periods. The necrotic layers did not completely disappear in the later periods of union, but were absorbed in particles in various parts of the graft union site. Similar observations on necrotic layers were also reported by many researchers such as Ünal and Özçağıran (1986), Tekintaş (1988), and Polat and Kaşka (1992).

# CONCLUSION

As a result, the development of the graft union was successful in all stages of the graft samples that developed sufficient callus tissue in the first 2 weeks after grafting. Early callus proliferation from rootstock and particularly scion two weeks after grafting was generally slow. The initial cambial differentiations in callus tissues were seen in sections 18 days after grafting. Cambial continuity between rootstock and scion



was established 32 days after grafting. The sections in the following periods exhibited that the graft partners were in vascular relationship. It was observed that the amount of callus tissue proliferated during the first two weeks after grafting directed the subsequent development of the union. In this respect, it is thought that developing methods that encourage callus formation in the early periods of the union will increase the success in related studies. By using rootstock and scion with very close thicknesses, well matching of graft partners from cambial zones affected the development of union positively and prepared a suitable basis for early vascular differentiation.

#### CONFLICT OF INTEREST

The author declare that he has no conflict of interest.

#### DECLARATION OF AUTHOR CONTRIBUTION

F.B: Conducting the research and writing the results were done by the author.

#### REFERENCES

- Adhikari, P.B., Xu, Q., & Notaguchi, M. (2022). Compatible graft establishment in fruit trees and its potential markers. *Agronomy*, *12*, 1981.https://doi.org/10.3390/agronomy12081981.
- Amsbury, S. (2022). Making a connection: cell-cell communication at the graft interface. *Plant Physiology*, 188, 19–21. https://doi.org/10.1093/plphys/kiab516.
- Asante, A.K., & Barnett, J. R. (1997). Graft union formation in mango (*Mangifera indica* L.). *Journal of Horticultural Science*, 72, 781-90. https://doi.org/10.1080/14620316.1997.11515571.
- Balta, F., Karadeniz, T., Tekintaş, F. E., & Şen, S. M. (1993, October 20-23). *Investigations on anatomical and histological development of the graft formation in chestnut (Castanea sativa)*. 1st Int. Congress on chestnut, Spoleto, Italy.
- Balta, F., Kazankaya, A., & Tekintaş, F. E. (1996a, Ocak 10-11). Kontrollu aşılama koşullarında bekletilen omega ceviz aşılarında aktarma öncesi anatomik ve histolojik gözlemler. Fındık ve Diğer Sert Kabuklu Meyveler Sempozyumu, Türkiye.
- Balta, F., Cangi, R., Doğan, A. & Karadeniz, T. (1996b). Rupestris Du Lot anacına aşılı iskenderiye misketi üzüm çeşidinde aşı kaynaşmasının gelişimi üzerine anatomik ve histolojik incelemeler. Van Yüzüncü Yıl Üniversitesi Ziraat Fakültesi Dergisi, 6(2), 201-208.
- Bijelić, S., Magazin, N., Džankić, S., Janković, D., Bogdanović, B. & Jaćimović, G. (2021). Advances in nursery production of hazelnut plants in Serbia – Successful grafting of different *Corylus avellana* L. cultivars and clones onto *Corylus colurna* L. rootstock. *Frontiers in Plant Science*, 12, 785015. doi: 10.3389/fpls.2021.785015.
- Cangi, R., Balta, F., & Doğan, A. (2000). Aşılı asma fidanı üretiminde kullanılan katlama ortamlarının fidan randıman ve kalitesi üzerine etkilerinin anatomik ve histolojik olarak incelenmesi. *Turkish Journal of Agriculture and Forestry*, 24, 393-398.
- Demirsoy, H., & Bilgener, Ş. (2006). Bazı uyuşur ve uyuşmaz şeftali/erik aşı kombinasyonlarında aşı yerinin anatomik olarak incelenmesi. *Anadolu Tarım Bilimleri Dergisi*, 21(1), 89-94.
- Dolgun, O., Tekintaş, F. E., & Ertan, E. (2008). A histological investigation on graft formation of some nectarine cultivars grafted on pixy rootstock. *World J. Agricultural Sciences*, 4 (5): 565-568.
- Farsi, M., Fatahimoghadam, M. R., Zamani, Z., Hassani, D., & Ahmadi, A. (2016). The histology of minigrafting of Persian walnut trees cv. chandler. *International Journal of Horticultural Science and Technology*, 3(2), 167-177. https://doi: 10.22059/ijhst.2016.62916.
- Goldschmidt, E. E. (2014). Plant grafting: new mechanisms, evolutionary implications. *Frontiers in Plant Science*, *5*, 727. https://doi.org/10.3389/fpls.2014.00727.
- Habibi, F., Liu, T., Folta, K., & Sarkhosh, A. (2022). Physiological, biochemical, and molecular aspects of grafting in fruit trees. *Horticulture Research*, *9*, uhac032. https://doi.org/10.1093/hr/uhac032.
- Hartmann, H. T. & Kester, D. E. (1974). Bahçe Bitkileri Yetiştirme Tekniği (Çevirenler: Kaska, N. & Yılmaz, M.). Çukurova Üniversitesi Ziraat Fakültesi, Yayın No: 79, Adana.
- Kalkışım, Ö., & Tekintaş F. E. (2011). Kızılcıkta (*Cornus mas* L.) aşı kaynaşması ile çelik köklenmesinin anatomik ve histolojik olarak incelenmesi üzerine bir araştırma. *Gümüşhane Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 1(2), 106-122.
- Kurotani, K. I., & Notaguchi, M. (2021). Cell-to-cell connection in plant grafting—molecular insights into symplasmic reconstruction. *Plant and Cell Physiology*, 62(9), 1362-1371. http://dx.doi.org/10.1093/pcp/pcab109.



- Kurt, H., & Tekintaş, F. E. (2020). Fıstık çamında (*Pinus pinea* L.) aşı kaynaşması ve çelik köklenmesinin anatomik ve histolojik olarak incelenmesi. *Euroasia Journal of Mathematics, Engineering, Natural & Medical Sciences*, 7(13), 135-148. doi.org/10.38065/euroasiaorg.422.
- Lagerstedt, H. B. (1981). A new device for hot-callusing graft unions fruit and nut trees. *Hortscience*, 16(4), 529-530. https://doi.org/10.21273/HORTSCI.16.4.529.

Lagerstedt, H. B. (1984). Hot-callusing pipe speeds up grafting. American Nurseryman, 160, 113-117.

- Mahunu, G. K., Adjei, P.Y., & Asante, A. K. (2012). Anatomical studies on graft formation in cashew (*Anacardium occidentale L.*). Agriculture and Biology Journal of North America, 3(4), 150-153. doi:10.5251/abjna.2012.3.4.150.153.
- Moore, R. (1981). Graft compatibility and incompatibility in higher plants. *Developmental & Comparative Immunology*, 5(3), 377-389. https://doi.org/10.1016/S0145-305X(81)80051-1
- Moore, R. (1984). A model for graft compatibility-incompatibility in higher plants. American Journal of Botany, 71(5), 752-758. https://doi.org/10.1002/j.1537-2197.1984.tb14182.x.
- Melnyk, C. W. (2016). Plant grafting: insights into tissue regeneration. Regeneration, 4(1), 3-14. https://doi.org/10.1002/reg2.71.
- Melnyk, C. W., & Meyerowitz, E. M. (2015). Plant grafting. Current Biology, 25(5), R183–188. https://doi.org/10.1016/j.cub.2015.01.029.
- Okay, Y., & Büyükkartal, N. (2001). Antepfistiğinda (Pistacia vera L.) farklı aşı yöntemlerinin anatomik yönden karşılaştırılması. The Herb Journal of Systematic Botany, 8(1), 73-82.
- Özdemir, B., Yılmaz, A., Büyükkartal, H. N., & Okay, Y. (2019). Anatomical analysis of graft compatibility in some almond scion-rootstock combination. *Journal of Agricultural Sciences*, 25(1), 29-37. https://doi.org/10.15832/ankutbd.538985.
- Pina, A., Errea, P., & Martens, H. J. (2012). Graft union formation and cell-to-cell communication via plasmodesmata in compatible and incompatible stem unions of Prunus spp. *Scientia Horticulturae*, 143, 144-150. https://doi.org/10.1016/j.scienta.2012.06.017.
- Polat, A. A., & Kaşka, N. (1992). Yeni Dünyalarda (*Eriobatrya japonica* L.) T, yama ve yongalı aşıların anatomik ve histolojik olarak incelenmesi. *Doğa Türk Tarım ve Ormancılık Dergisi*, 16(3), 529-541.
- Polat, M., Dolgun, O., Yıldırım, A., Aşkın, M. A., & Gökbayrak, Z. (2010). Graft union formation of spur apple varieties grafted on different rootstocks. *Journal of Food, Agriculture & Environment*, 8(2), 490-493.
- Seferoğlu, G., Tekintaş, F. E., & Özyiğit, S. (2004). Determination of grafting union success in 0900 Ziraat and Starks Gold Cherry cultivars on Gisela 5 ve SL 64 rootstocks. *Pakistan Journal of Botany*, 36(4), 811-816.
- Serdar, Ü., Köse, B., & Yılmaz, F. (2005). The structure of graft unions in European chestnut using different grafting methods. *HortScience*, 40(5), 1474–1477. https://doi.org/10.21273/HORTSCI.40.5.1474.
- Şenyurt, M. (2017). Corylus colurna L. anacına bazı fındık çeşitlerinin aşılanabilirliğinin incelenmesi (Doktora Tezi). Abant İzzet Baysal Üniversitesi, Bolu. https://tez.yok.gov.tr/UlusalTezMerkezi.
- Tekintaş, F. E. (1988). *Cevizlerde (Juglans regia* L.) *aşı kaynaşması ve aşı ile ilgili sorunlar üzerinde araştırmalar* (Doktora Tezi). Ege Üniversitesi, Bornova, İzmir. https://tez.yok.gov.tr/UlusalTezMerkezi.
- Tekintaş, F. E. (1991). Farklı anaçlar üzerine aşılanan turunçgil ve çeşitlerinde kaynaşmanın anatomik ve histolojik olarak incelenmesi üzerine araştırmalar. Van Yüzüncü Yıl Üniversitesi Ziraat Fakültesi Dergisi, 112, 68-81.
- Ünal, A. & Özçağıran, R. (1986). Göz aşısında aşı kaynaşmasının meydana gelişi üzerine bir araştırma. Doğa Türk Tarım ve Ormancılık Dergisi, 10(3), 339-407.
- Watanabe, S., & Nakazatomi, N. (1990). Formation of the graft union in apple T-budding. Bulletin of the Yamagata University. Agricultural Science, 2(1), 149-153.

134