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ORIGINAL ARTICLE

Histological Investigation of the Compatibility of *Prunus spinosa* as Interstock with Almond Cultivars

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Abstract

The aim of this study was to determine the early graft compatibility of 'Ferraduel', 'Ferragnes', 'Makako', 'Tarraco' and 'Vairo' almond cultivars with GF-677 rootstock and *Prunus spinosa* interstock. *P. spinosa*, which was used as an interstock on the GF-677 rootstock, was grafted using the whip grafting method, and almond cultivars were grafted on *P. spinosa* using the chip grafting method. Sections were taken 15, 30, 45, 60, 90 and 120 days after grafting for chip grafting and 150 days after grafting for whip grafting. Graft sections were examined histologically. The highest graft success was found in the *P. spinosa*/'Makako' combination with 96.66% and the lowest was found in the *P. spinosa*/'Ferraduel' combination with 83.33%. In the GF-677/*P. spinosa* combination, graft success was determined as 96.50%. Examination of the sections showed that callus formation started on the 15th day. It was found that the callus developed further on the 30th day and cambial differentiation started. On the 45th day, it was determined that the cambium connection between the graft elements was established and a distinct callus bridge was formed. On the 60th day, it was determined that the union between rootstock and scion was completed, callus tissue filled more than in the previous period and most of them turned into xylem cells. On the 90th day, it was determined that the newly differentiated cambium tissue continued its continuity along the entire graft surface and produced new conduction tissues, parenchymatic cells with a regular structure were formed between these tissues, and the newly formed conduction elements fulfilled their function between the graft elements. On day 120, graft union was determined to continue in most of the combinations and no signs of graft incompatibility were observed.

Keywords *Prunus dulcis* (Mill.) DA Weeb · GF-677 · Graft union · Incompatibility · Histology · Almond

Introduction

Graft incompatibility leads to the formation of unhealthy trees, breakage at the graft point, and early death of grafted trees (Zarrouk et al. 2006). The symptoms that cause these problems may take several years to appear, although they vary according to the graft combinations (Guclu and Koyuncu 2012). Therefore, morphological observation of incompatibility in a combination causes time and financial losses in fruit growing. Graft incompatibility is a critical issue for breeding rootstocks in fruit trees and the longevity of an orchard. For this reason, research on early detection of incompatibility has become a priority.

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In recent years, the use of clone rootstocks has become widespread. For this reason, the studies carried out by researchers to determine the best rootstocks in terms of graft compatibility with cultivars have accelerated. Graft incompatibility is a complex process that many researchers have investigated for years, but the mechanism has not been fully explained.

Almond seedling rootstocks, which are used as rootstocks, are suitable for gravelly soils with high lime content and water restriction, as well as increasing the growth strength, fruit yield and tree life of the grafted cultivar. However, almond rootstocks are susceptible to diseases such as root rot (*Rosellinia necatrix*), root cancer (*Agrobacterium tumefaciens*), and root rot nematodes (*Meloidogyne* spp.) (Aktan et al. 2020). On the other hand, peach seedling rootstocks are early fruiting (Soylu 2003), but unlike almond seedling rootstocks, they are not suitable for areas with high lime content and pH. Due to the incompatibility between almond and peach seedlings, swelling occurs in the upper part of the grafting point, and the trees decrease in yield at later ages.

Almond × peach hybrids (GF-) are very strong rootstocks and adapt well to poor soils since their root systems are well attached to the soil (Soylu 2003). GF-677, one of the first rootstocks that come to mind in almond cultivation, shows very good compatibility with almonds (Finn and Clark 2008; Coşkun 2012).

Plum seedlings are strong rootstocks used for almonds in heavy and moist soils that do not provide drainage conditions. The root systems of plum seedlings tend to be superficial, and the roots are usually less numerous and thicker than those of peaches or almonds. Plums are generally more tolerant of certain pathogens in heavy soils than almond and peach roots and are more resistant to heavy soil conditions with waterlogging. To utilize these advantages, plum seedlings can be used as rootstocks for almonds as well as interstock (Öylek Şahiner et al. 2019). Interstock is the plant part grafted between the rootstock and the cultivar. Interstock is used with different fruit species to alleviate tree size, incompatibility, fruit development and quality, and tree aging. Recent studies have shown that *P. spinosa* can be used as an interstock to alleviate disadvantages such as large trunk cross-sectional area and low yield in fruit growing (Öylek Şahiner et al. 2019; Miodragović et al. 2019). According to the study, *P. spinosa* interstock provided high density cultivation, early fruiting, wide soil adaptability, frost resistance, increased fruit size and better colouration (Maas et al. 2012; Milošević et al. 2011, 2015).

The main objectives of this study were: (1) to histologically investigate the compatibility of *P. spinosa* rootstocks with almond cultivars, which has not been previously investigated in the literature; (2) to determine the grafting success rates of *P. spinosa* seedlings with almond cultivars; (3) to examine the suitability of *P. spinosa*, known for its dwarf structure, resistance to external conditions, rapid and good compatibility in grafting, and adaptability to all conditions, as an interstock.

Materials and Methods

Plant Material

In the study, GF-677 was used as rootstock and *P. spinosa* was used as interstock. *P. spinosa* genotypes were taken from four different locations in Bolu province (Çivril neighbourhood, Atyaylası locality; around Abant lake; Mudurnu Çepni, Bulanık, Göncek villages; Göynük Hacımahmut village). 'Ferraduel', 'Ferragnes', 'Makako', 'Tarraco' and 'Vairo' almond cultivars were used as cultivars.

Practices of Graft and Collection of Graft Samples

In the experiment, *P. spinosa* scions were grafted on the GF-677 rootstock in the second week of March 2021 with the whip grafting method. In the same year, almond cultivars were grafted on *P. spinosa*, which was used as an interstock, in the first week of July with the chip grafting method. For each almond cultivar, 40 grafts were made. In order to follow the union of the grafts, three graft samples were taken by cutting 3 cm above and 3 cm below the grafting point at six different periods in chip grafting with *P. spinosa*. For microscopic examinations, graft samples were taken from *P. spinosa*/almond combinations 15, 30, 45, 60, 90 and 120 days after grafting and from GF677/*P. spinosa* combination 150 days after grafting. The tissues of the samples were fixed in formalin aceto-alcohol (FAA) in glass bottles with plastic lids to prevent deterioration.

Taking Graft Sections from Samples

After grafting, permanent preparations were prepared from the preserved graft samples using a rotary microtome (Leica RM 2125 RT, Nussloch, Germany) by taking sections with thicknesses between 15-40 µ. Sections taken 15 days after grafting were embedded in paraffin blocks. In the paraffin method, the method of Demirsoy and Bilgener (2006) was used for dehydration and paraffin infiltration. The number of procedures, duration and temperature levels during alcohol, xylol and paraffin applications during microwave procedures are shown in Table 1.

During the applications, the temperature of the material in the microwave reached 50–60 °C in ethyl alcohol treatments; 62–70 °C for xylol and its mixtures and paraffin treatments.

Staining and Examination of Graft Sections

Graft sections were stained with 1% safranin. Then, the permanent preparation was prepared after passing through a series of alcohol and xylol in order to both remove excess safranin and to obtain a better image (Kaçal 2004). These steps are shown in the following table (Coşkun 2012) (Table 2).

The state of union in the graft sections was examined under a microscope (Nikon Eclipse 80i, Nikon Corporation, Japan). In this examination, the formation of callus tissue between the graft pieces, the presence of necrotic layers, the establishment of cambium tissue and the formation of new vascular tissues, whether cambial continuity was achieved and the formation of new phloem and new xylem, and finally the continuity of wood tissue were taken into consideration (Göktaş et al. 2022; Bayram et al. 2014; Gür et al. 2020; Kurt and Tekintaş 2020).

Table 1 Process steps in paraffin method

Process applied	Duration and number of implementations
70% Ethyl alcohol	10 s, five
80% Ethyl alcohol	10 s, 10 s
90% Ethyl alcohol	10 s, 10 s
100% Ethyl alcohol	10 s, 10 s
Three parts ethyl alcohol +one part xylol	10 s, six
Two parts ethyl alcohol +two parts xylol	10 s, six
One part ethyl alcohol +three parts xylol	10 s, 10 s
Pure xylol	3 min, 10
Pure xylol + crumb paraffin	3 min, three
Add a layer of liquid paraffin	3 min, two
Liquid paraffin	3.5 min, two
Liquid paraffin	3.5 min, 40–50
(modified each time)	–

Table 2 Staining and permanent preparation steps

Number of transactions	Application	Duration
1	1% Safranin	4 min
2	70% Ethyl alcohol	Until the excess paint is washed off
3	80% Ethyl alcohol	Until the excess paint is washed off
4	90% Ethyl alcohol	Until the excess paint is washed off
5	100% Ethyl alcohol	4 min
6	Xylol	–
7	Coating with Entellan	–

Results and Discussion

A total of 150 chip graftings were performed, 135 of which were successful with a rate of 89.99% (Table 3). In *P. spinosa*/almond combinations, 'Makako' cultivar had the highest grafting success rate with 96.66%, followed by 'Tarraco' (93.33%), 'Ferragnes' (90%), 'Vairo' (86.66%) and 'Ferraduel' (83.33%). In the *P. spinosa* combination grafted on GF-677 rootstock as an interstock, grafting success was determined by counting the number of viable chip eyes. A total of 200 whip grafting was performed and 96.50% grafting success was achieved. Uğur and ve Kargı (2018) grafted apricot cultivars on Myrobolan 29C and GF-677 rootstocks with clonal *P. spinosa* genotypes obtained by selection. The graft success rates of the combinations varied between 47.33 and 96.66%, and the graft success rates of *P. spinosa* combinations were significantly lower than the other rootstocks. The fact that very different results were obtained from the studies on graft compatibility is due to the difference in the biochemical relationship between rootstock and cultivar (Asma and Birhanlı 2000). The results obtained were more successful than previous studies. This may be due to the genetic variation of the rootstock, soil conditions and climatic conditions (Dimitrova and Marinov 2002) as well as the combinations used.

The State of Union in *P. Spinosa*/Almond Combinations

In all *P. spinosa*/almond combinations, a weak callus tissue was observed along the cutting surface. Callus tissue was observed to be denser in *P. spinosa* than in the cultivar. In graft combinations, it was observed that the callus tissue formed in the graft junction area originated from the xylem

Table 3 Grafting method and success rates in chip and whip grafting

Rootstock	Cultivars	Graft method	Graft success rate (%)
P. spinosa	'Ferraduel'	Chip grafting	83.33
	'Ferragnes'		90.00
	'Makako'		96.66
	'Tarraco'		93.33
	'Vairo'		86.66
–		*Total*	*89.99*
GF-677	*P. spinosa*	Whip grafting	96.50

of the rootstock and the cambium cells consisting of meristematic cells of the scion. On the 15th-day graft sections, no textural differences were observed between the cultivars. The weak connection between the graft elements also made it difficult to cut the graft samples at micron thickness, so the samples were placed in paraffin blocks but still could not be sectioned. For this reason, 15th-day graft samples were examined and interpreted at micro- and macro-levels. Weak callus formation can be caused by weak grafting and improper care conditions before and after grafting (Ünal and Özçağıran 1986). Balta and Şen (2023) and Karadeniz (1998) reported that callus formation was observed 14 days after grafting, but cambial differentiation did not start. In similar studies carried out by researchers in different fruit species, it was reported that the first cambial formation started with callus tissue 14–15 days after grafting, but cambial differentiation had not started yet (Kankaya et al. 2001; Coşkun 2012; Kalkışım and Tekintaş 2011; Karadeniz and Bornman 2003; Tekintaş et al. 1988; Tekintaş 1991; Yıldız 1997), and callus formation was more intense in the rootstock compared to the cultivar (Şenyurt 2017; Göktaş et al. 2022).

Sections taken after 30, 45, 60, 90 and 120 days in the *P. spinosa*/'Ferraduel' combination are shown in Fig. 1. In *P. spinosa*/'Ferraduel' combination, callus tissue was determined to have thin membrane, high turgor and parenchymatic structure in 30th-day sections. It was determined that more callus tissue was produced from the rootstock than the scion, new xylem and phloem tissue continued to be produced from the combined cambium of the rootstock and scion, and conduction bundles started to form in the new xylem tissue. During pre-graft preparation, areas where cell death occurs occur on the cut surfaces. These are observed as necrotic areas but are often absorbed by the callus layer formed by the division of parenchymatic cells (Davies et al. 2017). On the 45th and 60th day, it is observed that the callus bridge is clearly established, and differentiation continues and parenchymatic cells continue to appear and mature

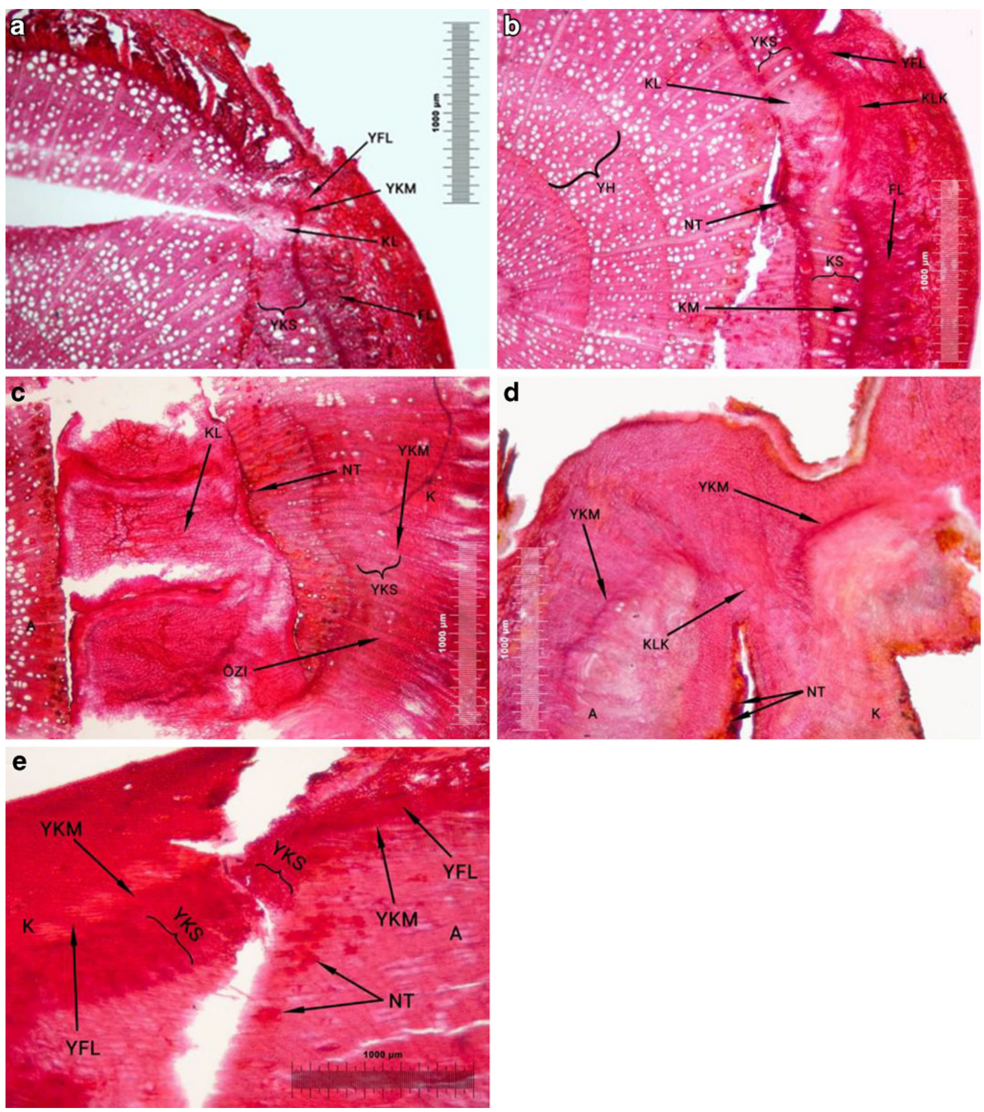

Fig. 1 The appearance of tissues in cross-section taken 30 (**a**), 45 (**b**), 60 (**c**), 90 (**d**) and 120 (**e**) days after grafting in the *P. spinosa*/'Ferraduel' graft combination. (Safranin, 4 × 0.10) *A* Rootstock, *K* Scion, *FL* Phloem, *KS* Xylem, *KM* Cambium, *KL* Callus, *NT* Necrotic layer, *YFL* New phloem, *YKS* New xylem, *YKM* New cambium, *OZI* Pith rays, *PAH* Parenchymatic cells, *VD* Vascular tissue, *CLC* Callus bridge, *YH* Annual ring

in the new xylem tissue. Necrotic layers continue to exist towards the inner part of the graft section. Yıldız (1997), in his study in which the effect of different graft eyes on graft success in walnut was examined, reported that new cambium was established in the callus tissue formed from the graft scion on the 40th day, conduction bundles were formed from this cambium, cambial continuity was ensured and necrotic layers continued to be seen on the cut surface of the scion. In the study by Sesli and Tekintaş (2020), in which they grafted the 'Chandler' cultivar onto 'Chandler' and 'Fernor' seedlings, they reported that union between the graft elements continued on the 60th day, callus was established, necrotic areas were present in some places, vascular connection was established with the presence of conduction bundles and union was achieved. On the 90th-day sections, it was observed that there was an excellent union on the outer junction surfaces, the gap between the rootstock and the scion could not be completely filled, necrotic layers still existed, but necrosis in the callus tissue disappeared on the 120th day. Göktaş et al. (2022) examined the compatibility of almond genotypes and 'Ferragnes' almond cultivar on peach rootstock and reported that reported that vascular differentiation continued in all of the combinations in the sections taken on the 90th day after grafting, and although cuts were observed in the cambium layer, continuity was maintained in this area. On the 120th day, it was recorded that all stages of graft union occurred, the newly differentiated cambium tissue continued its continuity along the graft surface and produced new conduction tissues. Çetinbaş et al. (2018) examined the compatibility of some apricot and plum cultivars grafted on 'Rootpac' series rootstocks and reported that callus formation was determined in all 120th-day sections, necrotic breaks started although their intensity was different, cambial differentiation was established and cambial continuity was ensured.

Sections taken after 30, 45, 60, 90 and 120 days in the *P. spinosa*/'Ferragnes' combination are shown in Fig. 2. In the *P. spinosa*/'Ferragnes' combination, it was observed that

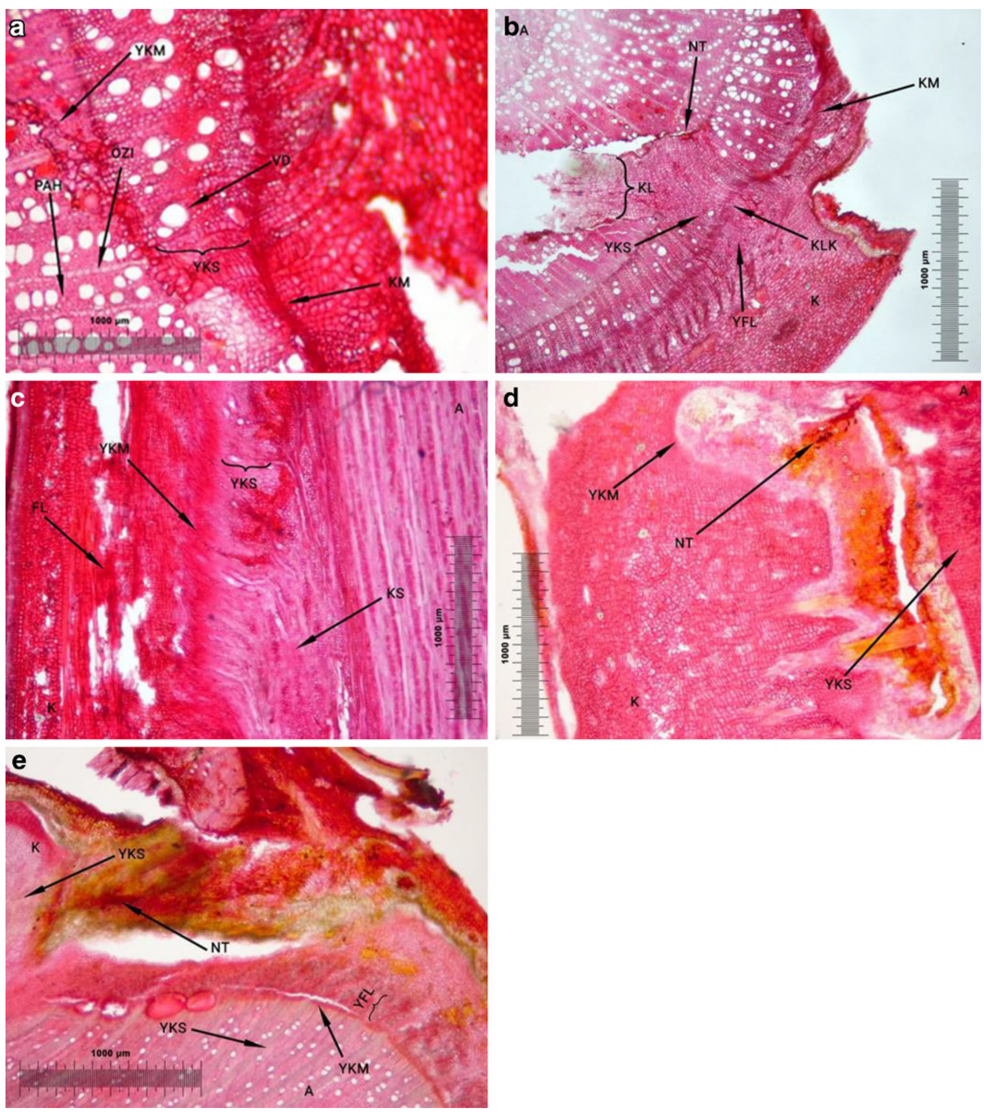

Fig. 2 The appearance of tissues in cross-section taken 30 (**a**), 45 (**b**), 60 (**c**), 90 (**d**) and 120 (**e**) days after grafting in *P. spinosa*/'Ferragnes' graft combination. (Safranin, 4× 0.10) *A* Rootstock, *K* Scion, *FL* Phloem, *KS* Xylem, *KM* Cambium, *KL* Callus, *NT* Necrotic layer, *YFL* New phloem, *YKS* New xylem, *YKM* New cambium, *OZI* Pith rays, *PAH* Parenchymatic cells, *VD* Vascular tissue, *CLC* Callus bridge, *YH* Annual ring

a very successful union occurred 30th days after grafting, a significant amount of callus tissue was produced from the xylem tissue of the rootstock, and the new cambium cells formed in it were bent and curved according to the state of overlap. It was observed that this new cambium formed from the scion continued along the callus cells at the side junctions and merged with the original cambium of the rootstock. It was determined that the rays of the pith, which start from the pith and extend towards the phloem, and their extensions in the rootstock are joined in places with their extensions in the scion. Göktaş et al. (2022) examined the compatibility of peach and almond genotypes and 'Ferragnes' almond cultivar and reported that callus density increased in the sections taken on the 30th day and xylem cells were formed in the callus. The researcher also stated that necrotic areas were very few in the graft samples examined. It was reported that a cambium connection was established between the rootstock and scion on the 45th day, and the union between the rootstock and scion was achieved on the 60th day. Cangi et al. (2000) used 420A, 5BB and 99R as rootstocks and 'Alphonse Lavellee', 'Çavuş', 'Erciş', 'Gamay Noir A Jus Blanc', 'Razakı' and 'Yuvarlak Çekirdeksiz' grape cultivars as scions and reported that there was a curved vascular relationship in the sections taken on the 45th day, that more vascular tissue was seen in the 5BB rootstock combination, and that cambial activity was established in the 420A rootstock. It was determined that callus tissue was produced more between rootstock and scion compared to the previous period, most of the tissues were transformed into xylem cells, necrotic layers trapped in the tissues transformed into xylem cells were observed and these layers decreased in volume in 90th-day sections. Coşkun (2012) reported in his study on apricot cultivars grafted on clone rootstocks that the callus tissue maintained its density throughout the grafting site in the 60th-day sections, necrotic layers continued to exist in the callus tissue in the combinations, but they were not at a level that affected the union. Azimi et al. (2015) investigated graft union and incompatibility in 'Memecik' and 'Nizip Yağlık' cultivars grafted on 'Gemlik' rootstock and found that cambium cells were formed and undifferentiated parenchymatic cells were found in 90th-day sections. The newly differentiated cambium tissue produced new conduction tissues along the graft surface. It has been observed that conduction bundles are formed in parenchymatic cells, which have a regular structure in these tissues. In 120th-day sections, it was determined that the rootstock and scion were unions, but the cavity could not be filled by callus cells in the inner parts of the union areas. It was also observed that the union was healthy on the outer side of the union zone, new xylem and phloem cells continued to be produced from the cambium cells, and the cells in the new xylem tissue differentiated into parenchymatic cells. Koyuncu et al. (2007) examined the compatibility of 'St. Julien A'/'Red Globe' and 'St. Julien A'/'Fantasia' nectarine combinations and reported that callus bridge was established between the grafting elements and cambial differentiation gained continuity in 120th-day sections.

Sections taken after 30, 45, 60, 90 and 120 days in the *P. spinosa*/'Makako' combination are shown in Fig. 3. In the *P. spinosa*/'Makako' combination, callus tissue was determined to have thin membrane, high turgor and parenchymatic structure in 30th-day sections. It was also found that there were significant necrotic layers on the grafting cross-sectional surface of the rootstock and scion, but they did not prevent union. Özdemir et al. (2019) investigated the graft compatibility of 'Lauranne' almond cultivar on different rootstocks and found that callus tissue was formed in the 30th-day sections, but cambium union did not occur. In the 45th-day sections, it was determined that a large amount of callus tissue was produced from the cambium and parenchyma cells of the rootstock. On the 60th and 90th days, it was observed that the callus bridge became more prominent and continued to produce new xylem and phloem tissues by providing union. Şenyurt (2017) reported in study on *Corylus colurna* that new cambium cells formed from rootstock and scion were seen bent and curved in 40th-day sections, new vascular connections started to be formed in these cells, cambial continuity was ensured and necrotic layers, which were observed intensively before, weakened in this period. Göktaş et al. (2022) examined the compatibility of almond genotypes and 'Ferragnes' almond cultivar on peach rootstock and reported that there were necrotic areas in the tissues close to the old xylem in the 60th-day sections. On 120th-day sections, it was determined that the union continued, the cambium of the rootstock and the scion were united in a healthy way, and they continued to produce new phloem and xylem tissues. During this period, new xylem and phloem and new cambium tissues were clearly seen and these tissues were in the form of regular cells. Demirsoy and Bilgener (2006), in their study on graft compatibility in incompatible peach/plum combinations, reported that cambium formation was interrupted but completed in 120th-day sections. It was also reported that callus and cambium formation and vascular differentiation occurred.

Graft sections taken after 30, 45, 60, 90 and 120 days in the *P. spinosa*/'Tarraco' combination are shown in Fig. 4. In the *P. spinosa*/'Tarraco' combination, it was determined that callus cells filled the gap at the side junctions of the rootstock and scion in the 30th-day sections. It was determined that there were fragmented necrotic layers on the graft section surfaces. On the 45th-day sections, it was determined that bent and curved new cambium cells in the callus tissue, which started to form a regular structure, provided cambial continuity between the rootstock and the scion. It has

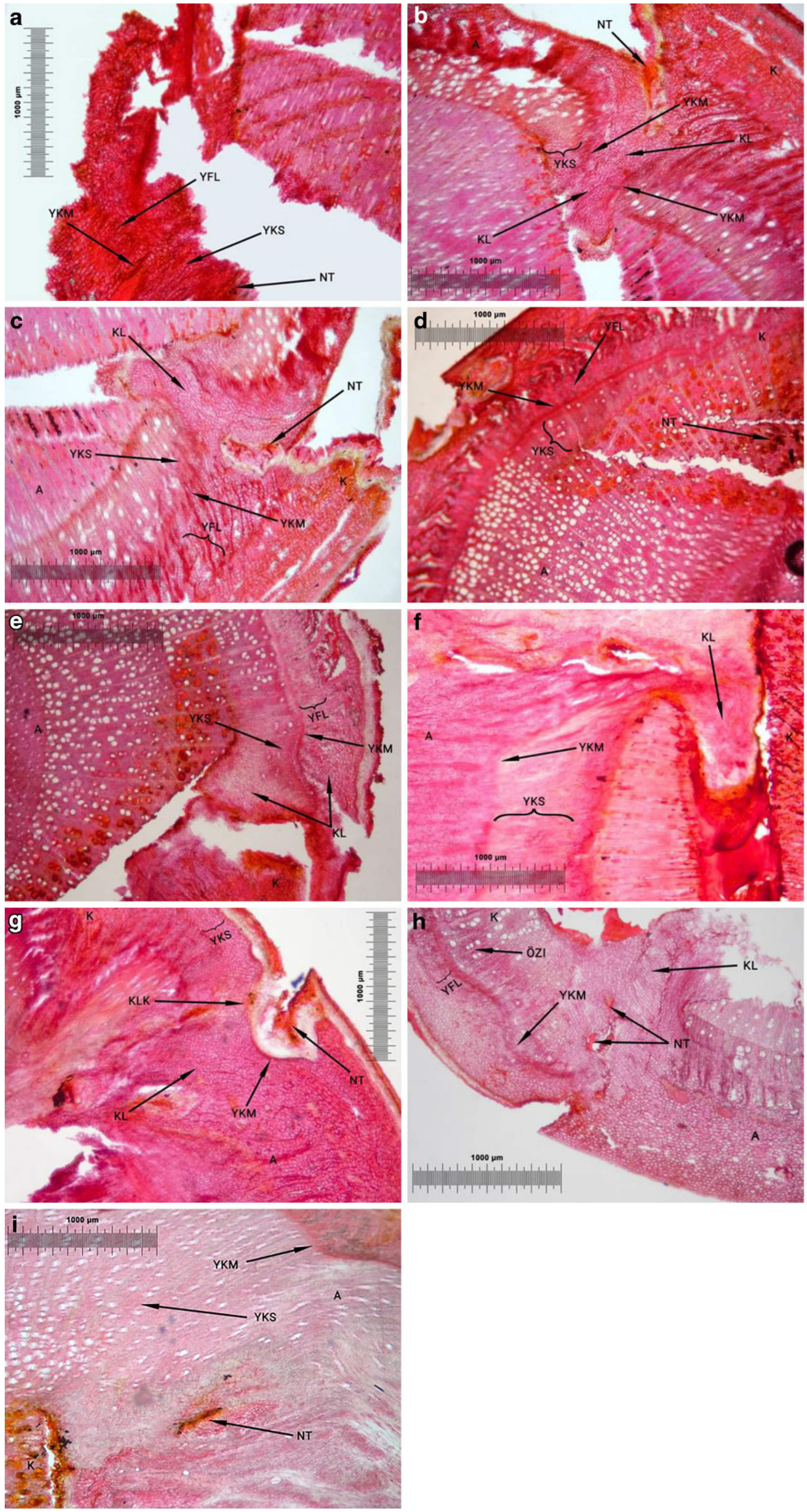

Fig. 3 The appearance of tissues in cross-section taken 30 (**a**), 45 (**b**, **c**), 60 (**d**, **e**), 90 (**f**, **g**) and 120 (**h**, **i**) days after grafting in the *P. spinosa*/'Makako' graft combination. (Safranin, 4× 0.10) *A* Rootstock, *K* Scion, *FL* Phloem, *KS* Xylem, *KM* Cambium, *KL* Callus, *NT* Necrotic layer, *YFL* New phloem, *YKS* New xylem, *YKM* New cambium, *OZI* Pith rays, *PAH* Parenchymatic cells, *VD* Vascular tissue, *CLC* Callus bridge, *YH* Annual ring

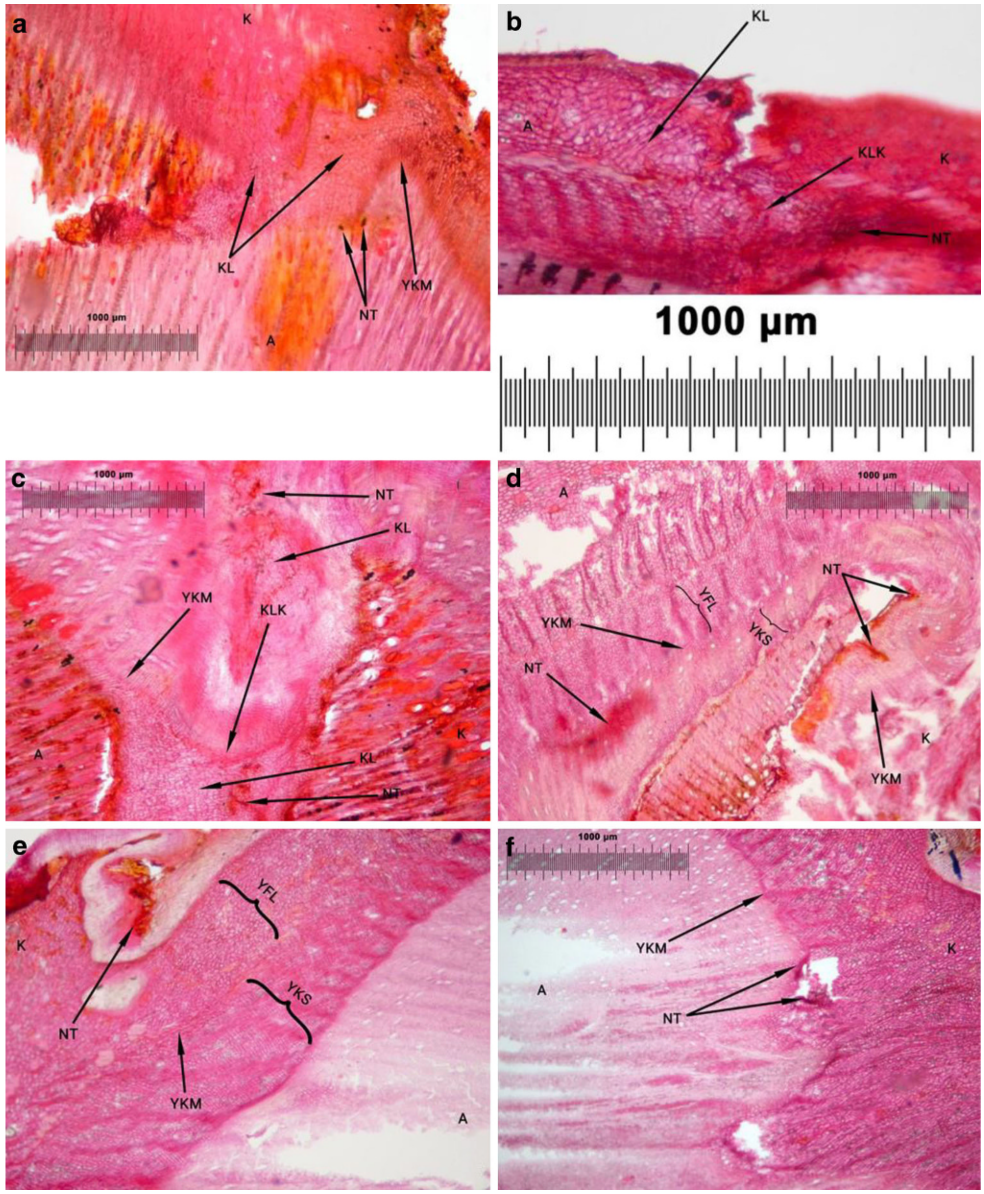

Fig. 4 The appearance of tissues in cross-section taken 30 (**a**), 45 (**b**), 60 (**c**), 90 (**d**) and 120 (**e**, **f**) days after grafting in the *P. spinosa*/'Tarraco' graft combination. (Safranin, 4× 0.10) *A* Rootstock, *K* Scion, *FL* Phloem, *KS* Xylem, *KM* Cambium, *KL* Callus, *NT* Necrotic layer, *YFL* New phloem, *YKS* New xylem, *YKM* New cambium, *OZI* Pith rays, *PAH* Parenchymatic cells, *VD* Vascular tissue, *CLC* Callus bridge, *YH* Annual ring

been determined that newly formed cambium cells begin to establish vascular connections by forming phloem and xylem. Tekintaş et al. (1988) reported that cambial continuity occurred 40 days after grafting in walnut. In the 60th- and 90th-day sections, it was determined that the callus tissue filled the gap between the rootstock and the pencil compared to the previous period and the union between the graft elements was successfully realized. During this period, it was determined that new conduction bundles continued to develop in the callus tissue. Necrotic layers were observed to continue to exist in some places. Şenyurt (2017) reported that in the 60th-day sections of *C. colurna*, there was a successful union between the graft elements, the callus tissue gained a parenchymatic structure, the necrotic structure weakened, conduction bundles were formed from the new cambium formed straight and curved, and vascular connection was observed. Bayram et al. (2014) investigated the compatibility of 'Bacon', 'Zutano', 'Fuerte' and 'Hass' avocado cultivars on 'Mexicola' and 'Topa' rootstocks and reported that a successful union occurred in the 90th-day sections, necrotic areas continued to exist in the rootstock xylem, callus formed and filled between the grafting elements. It was determined that the graft union observed in the previous periods (120th-day) was maintained in this period, and new xylem and phloem and new cambium tissues were observed significantly compared to the previous periods. In studies conducted by researchers in different fruit species, it was reported that callus tissue had a parenchymatic structure in the graft sections examined 120 days after grafting, cambial differentiation continued between the

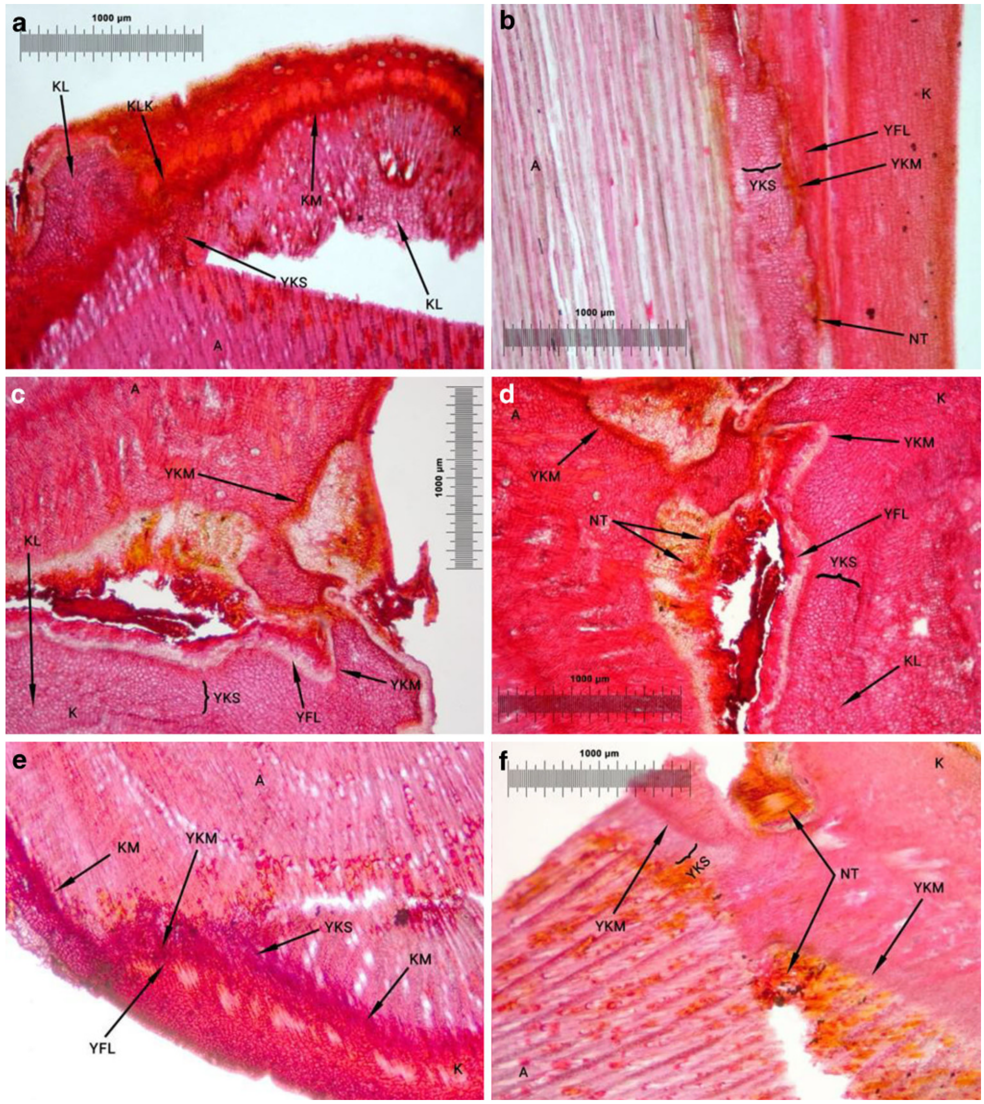

Fig. 5 The appearance of tissues in cross-section taken 30 (**a**), 45 (**b**), 60 (**c**, **d**), 90 (**e**) and 120 (**f**) days after grafting in the *P. spinosa*/'Vairo' graft combination. (Safranin, 4 × 0.10) *A* Rootstock, *K* Scion, *FL* Phloem, *KS* Xylem, *KM* Cambium, *KL* Callus, *NT* Necrotic layer, *YFL* New phloem, *YKS* New xylem, *YKM* New cambium, *OZI* Pith rays, *PAH* Parenchymatic cells, *VD* Vascular tissue, *CLC* Callus bridge, *YH* Annual ring

graft elements, vascular connection was established with the formation of xylem and phloem, and nectoric layers continued but decreased compared to previous periods (Dolgun et al. 2008; Koyuncu et al. 2007).

Graft sections taken after 30, 45, 60, 90 and 120 days in the *P. spinosa*/'Vairo' combination are shown in Fig. 5. In the *P. spinosa*/'Vairo' combination, callus cells were observed intensively at the side joints of the graft elements in the 30th-day sections, but it was determined that they could not fill the middle parts of the graft yet. In the 45th- and 60th-day sections, it was determined that the callus cells continued to fill the gap between the rootstock and the scion, the necrotic layers were broken down by the callus tissue at the side joints, but continued towards the middle parts of the graft and did not affect the union negatively. It was determined that the callus formed at the graft union sites began to exhibit a regular structure and the presence of xylem and phloem cells became more prominent. It was found that new parenchymatic cells started to develop for the new xylem tissue. It was determined that the cambium tissue differentiated between callus cells and merged to provide cambial continuity between rootstock and scion. Tekintaş and Dolgun (1996) examined the graft compatibility of peach and nectarine cultivars grafted on almond seedlings and reported that cambial connection was established, new conduction bundles started to form, vascular connection was realized and necrotic layers decreased in 45th-day sections. In the 90th-day sections, it was determined that the gap between the rootstock and the scion was filled in some combinations, but not completely in others. However, in graft samples where callus tissue did not fill the entire surface, it was determined that sufficient callus tissue formed at the side union did not affect the continuity of the cambial connection established in the previous period. It was determined that the callus tissue gained a parenchymatic structure consisting of regular cells and formed new

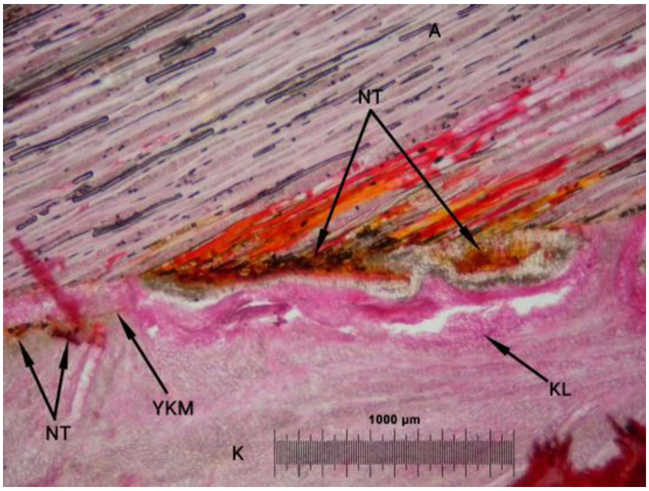

Fig. 6 The appearance of tissues in cross-section taken 150 days after grafting in the GF-677/*P. spinosa* graft combination. (Safranin, 4× 0.10) *A* rootstock, *K* Scion, *KL* Callus, *NT* Necrotic layer, *NCC* New cambium

transmission tissues at the junctions. Polat et al. (2010) examined the graft development of 'Scarlet Spur', 'Red Spur' and 'Redchief' apple cultivars grafted on M7, M16, Pj80 rootstocks and reported that all of the combinations were compatible, sufficient callus tissue was formed, good cambial development and vascular connection were realized in about 90 days after grafting. In 120th-day sections, it was observed that graft union continued successfully. It was determined that the developments that occurred in the previous period became more evident in this period. Göktaş et al. (2022) examined the compatibility of some almond genotypes and 'Ferragnes' almond cultivar on peach rootstock and reported that callus bridge was formed between the graft elements in all of the graft combinations 120 days after grafting, cambial differentiation gained continuity and no negativity was observed in terms of graft union in the combinations.

State of Union in the GF-677/*P. Spinosa* Combination

In the sections taken on day 150 of GF-677/*P. spinosa* graft combination (Fig. 6), it was found that callus cells, mostly from the cambium of the rootstock and partially composed of parenchyma cells, filled the gap between the rootstock and the scion at a very good level. It was determined that necrotic layers remained within the callus cells, but did not have any effect on the union of the rootstock and scion. It was determined that the pith rays developing towards the phloem continue to develop in a regular manner by merging with the pith rays in the scion. It was determined that the callus bridge was established, cambial connection was established and new xylem and phloem tissues continued to be produced.

Ada and Ertan (2013) examined the anatomical development of the graft union between chestnut (*Castanea sativa* Mill.) and oak (*Quercus* sp.) and reported that graft success was obtained only from the splice grafting method. In 30th-day sections, it was reported that callus tissue started to form, but cambial continuity occurred 150 days after grafting. Azimi et al. (2015), in a histological study to determine graft incompatibility in olives, reported that differentiation was established in air pockets and wound tissues were found in these areas in sections taken on day 180. It also reported that necrotic areas were observed in the form of lines, conduction bundles and sclerenchyma cells were formed in the cambial area. Ksia et al. (2016) histologically examined the graft compatibility of 'Achaak' and 'Porto' grafted almond cultivars on GF-677 and Garnem rootstocks. In sections taken at 150 and 180 days after grafting, it was reported that there was no continuity of cells in the Garnem/'Achaak' combination and that there was incompatibility between rootstock and scion due to poor development. In the GF-677/'Achaak' combination, it was reported that cambium cells were organized and homogeneous at the graft union and no incompatibility was detected in this combination. Balta (2023) examined the anatomical and histological development of graft union in the bladeless grafting technique in hazelnut (*Corylus avellana* L.) and reported that union progressed successfully in 140th-day sections. The researcher also reported the presence of fragmented and absorbed necrotic layers in different parts of the union.

Conclusion

In this study, the graft compatibility of *P. spinosa* with 'Ferraduel', 'Ferragnes', 'Makako', 'Tarraco' and 'Vairo' almond cultivars was investigated. As a result, no signs of incompatibility were found in histological examinations of graft samples taken at different times. Necrotic layers seen in the combinations may be related to graft errors and maintenance conditions, and it is recommended that *P. spinosa*, which is used as an interstock between rootstock and cultivar, should be examined in the long term to use these combinations successfully in the future. The results of the study are valid for 4-month samples. For this reason, it should be kept in mind that combinations that do not show problems in graft compatibility may show late incompatibility in the future.

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Conflict of interest L. Kirca and T. Karadeniz declare that they have no competing interests.

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