



Early detection of graft-incompatibility in hawthorn (*Crataegus azarolus* L.) trees on apple, pear, and quince rootstocks

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Abstract

Aim of study: This study was conducted to determine the usability of some clonal rootstocks of apple (MM 106 and MM 111), pear (Fox 11), and quince (Quince A) for hawthorn trees propagation.

Area of study: Fruit Research Institute, Isparta and Hatay Mustafa Kemal University, Hatay, Türkiye.

Material and methods: ‘Sultan’ hawthorn cultivar was budded on the following clonal rootstocks: pear Fox 11, quince A, and apple MM 106 and MM 111. Plants of hawthorn seedlings (*Crataegus azarolus* L.) budded with ‘Sultan’ cv. were used as control. External visual diagnosis of the scion-rootstock graft combinations was performed by observing visual symptoms in budded trees growing in nursery conditions, as well as anatomic and histological investigations of the incompatibility in the laboratory.

Main results: In this study, healthy scion development and callus tissue in the graft region were formed in the seedling rootstock as well as on Fox11 and Quince A clonal rootstocks. In contrast, MM 106 and MM 111 apple rootstocks showed incompatibility symptoms, with insufficient scion vigor and unstructured callus tissue development.

Research highlights: ‘Translocated’ and ‘located’ graft incompatibility symptoms were observed in Sultan/MM 106 and Sultan/MM 111 combinations. Further studies are necessary to confirm the early good compatibility found in nursery conditions, testing the effect of those clonal rootstocks from different species (Fox11 and Quince A) on vigor, yield, and fruit quality traits in orchard conditions.

Additional key words: clonal rootstock; histology; vigor; translocated incompatibility; localized incompatibility.

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Introduction

Türkiye is one of the origin areas of hawthorn and contains more than 30 hawthorn species (*Crataegus* spp.) in different ecological regions. These regions have at least one primary species and other secondary or several common species with local genotypes (Ercisli *et al.*, 2015; Caliskan *et al.*, 2016; Donmez & Ozderin, 2019). The hawthorn fruits are commonly consumed either in fresh or processed form such as juice and jam, due to their charac-

teristic flavor (astringent, sweet, and floral). In addition, a high content of several macro- and micro-elements, and beneficial effects on health have been reported (Gundogdu *et al.*, 2014; Caliskan, 2015; Wu *et al.*, 2020). The ‘Sultan’ hawthorn cultivar (*Crataegus azarolus* L.) has been successfully released due to its large fruit, high eating quality, and rich aroma compounds (Çalışkan *et al.*, 2018; Dursun *et al.*, 2021), reaching high prices in the local markets. It is grown in regular orchards for fresh fruit consumption in the eastern Mediterranean region of Türkiye, and new

orchards are currently established with this cultivar in different regions of Türkiye.

Many hawthorn species are grown for their edible fruits in Asia (Guo & Jiao, 1995; Li *et al.*, 2015), Central and South USA (Payne & Krewer, 1990; Velasco-Hernández *et al.*, 2017), and the Mediterranean countries (Bahri-Sahloul *et al.*, 2009; Caliskan *et al.*, 2016; Issaadi *et al.*, 2020). Similarly, the hawthorn species with medicinal value and high fruit quality cause an increase in hawthorn cultivation in those areas. However, one of the most important limiting factors for hawthorn tree cultivation is propagation. Vegetative propagation of hawthorns by cuttings is not a useful technique because of their poor rooting ability. Similarly, difficulties in germination due to the seed coat thickness and deep dormancy in seedling rootstock production, slow growth of plants after germination, and a prolonged juvenile period due to its heterozygote genetic structure, are also important problems for hawthorn propagation and cultivation (Bayazit *et al.*, 2018; Çalışkan *et al.*, 2020). Nevertheless, grafting onto seedling rootstocks is mainly used for the propagation of hawthorns (Hartmann *et al.*, 2002). In fact, Caliskan & Karaman (2018) showed that whip grafting onto hawthorn seedling rootstock is highly successful in March and April months in the ecological conditions of the Eastern Mediterranean region of Türkiye. Propagation by seeds (Çalışkan *et al.*, 2020) or tissue culture (Nas *et al.*, 2012) could be preferred for rootstock production, but it takes at least 2 or 3 years for producing a young tree to be grafted and planted in orchard conditions. Until now, there is no detailed research on the determination of a specific clonal rootstock allowing clonal propagation of hawthorn trees.

Hummer & Janick (2009) mentioned that hawthorn species could be closely related to the genera *Pyrus*, *Cydonia*, and *Malus*. Therefore, investigating the suitability of the available clonal rootstocks from these species could be considered a very interesting strategy for the spread of cultivation of hawthorn trees. It is commonly accepted that the greater is the taxonomic distance between the rootstock and scion, the chance of successful graft union decreases (Flaishman *et al.*, 2008). Interspecific grafting between rootstock and scion belonging to different species of the same genus, can be compatible in certain cases but intrafamilial grafts are rarely compatible (Andrews & Marquez, 2010; Goldschmidt, 2014). Furthermore, successful examples of good graft compatibility between different species are well known in peach/plum (Zarrouk *et al.*, 2010), pear/quince (Esen *et al.*, 2005; Machado *et al.*, 2016), apricot/plum (Reig *et al.*, 2018a; Gür *et al.*, 2020), and European and Japanese plums on peach × almond hybrids and different plum species (Reig *et al.*, 2018b, 2019) graft-combinations.

Graft compatibility or incompatibility can be identified as success or failure at the point of graft union between rootstock and scion (Andrews & Marquez, 2010) and the presence of other abnormalities on the aerial part of the

grafted tree (Amri *et al.*, 2021). Different anatomical, physiological and biochemical interactions occur in the grafting union and above it, in the budded scion (Yildirim *et al.*, 2010; Reig *et al.*, 2018a, 2019; Amri *et al.*, 2021). After successful grafting, callus formation, the establishment of a continuous cambium, the creation of new vascular tissue, and the production of a functional vascular system at the grafting location appear (Melnik, 2017; Balbi *et al.*, 2019). Also, the survival rate and the quality of vascular connections formed in the graft union primarily affect the success of grafting (Reig *et al.*, 2018a).

Graft incompatibility is commonly classified into two groups, known as ‘translocated’ and ‘localized’ types (Andrews & Marquez, 2010; Reig *et al.*, 2018a). The ‘translocated’ type is characterized by visual symptoms such as early termination of the scion, shriveling, leaf chlorosis, premature leaf dropping, and a radicular system not fully developed in the sapling (Zarrouk *et al.*, 2006; Goldschmidt, 2014). The ‘localized’ type is characterized by anatomical irregularities at the graft union interface and breaks of vascular tissue in the callus bridge (Hartman *et al.*, 2002; Zarrouk *et al.*, 2010; Reig *et al.*, 2019). Several phenotypic, anatomical, and enzymatic methods have been studied to determine grafting incompatibility in fruit species such as apple (Dolgun *et al.*, 2009; Yildirim *et al.*, 2010), pear (Gulen *et al.*, 2002), apricot (Reig *et al.*, 2018b), peach and nectarine (Zarrouk *et al.*, 2006; 2010; Amri *et al.*, 2021), and kiwifruit (Li *et al.*, 2021). However, to our best knowledge, there is no study on the incompatibility of hawthorn trees searching for those two types of graft-incompatibility. In addition, graft compatibility or incompatibility can be phenotyped by anatomical evaluation one year after grafting (Reig *et al.*, 2019; Irisarri *et al.*, 2021), although delayed cases of incompatibility can be found in both types of graft-incompatibility: ‘translocated’ (Moreno *et al.*, 1993) and ‘localized’ (Reig *et al.*, 2019).

This study aimed to investigate the graft compatibility between hawthorn and four different pome fruit tree species and rootstocks. The obtained results will contribute to the application of modern fruit tree growing techniques in hawthorn cultivation by determining if clonal rootstock(s) from different species can be used in the production of hawthorn trees.

Material and methods

Plant material

This study was carried out in the experimental research area and laboratories of the Fruit Research Institute, Eğirdir/Isparta (37°49’19” N, 30°52’23” E, and altitude: 900 m), Türkiye. Two clonal rootstocks of apple (*Malus communis* L.) (MM106 and MM111), one of quince (*Cydonia oblonga* Mill) (Quince A), and one of pear (*Pyrus communis* L.)



Figure 1. Fruits, seeds, and fruit set in bearing trees of the ‘Sultan’ hawthorn cultivar.

(Fox11), which could be genetically related to hawthorn species in the *Maloideae* subfamily (Hummer & Janick, 2009), were used as hawthorn rootstocks. These rootstocks were chosen because of their semi-vigorous growth, tolerance to heavy soil conditions, and suitability for calcareous soils with high pH. One-year-old hawthorn (*C. azarolus* L) saplings were also assessed as control. The ‘Sultan’ hawthorn cultivar was used as the budded scion for all of those rootstocks (Fig. 1). This scion cultivar is popular for its big fruit size (~15 g) and sweet-sour fruit flavor. At ripening, its yellow-green fruits contain 2-4 seeds, ~15% total soluble solids, and ~1.4% titratable acidity (Çalışkan *et al.*, 2018).

Grafting and grafting performance

One-year-old rooted plants of rootstocks were planted directly into the soil with planting spaces of 15 cm x 30 cm in February 2020, at the nursery conditions under a plastic greenhouse. Buds of the ‘Sultan’ hawthorn cultivar were taken from a producers’ orchard in the Belen district of the Hatay province (36°43’09” N, 36°13’80” E, and altitude: 812 m), from young shoots of the current vegetative period in 2020. The shoots were stored at 4 °C until they were used in Eğirdir/Isparta, to avoid loss of their moisture.

The ‘Sultan’ cultivar was T-budded *in situ* in September 2020 on the five assessed rootstocks with three replicates, each consisting of ten plants for each budding combination. The ratio of graft success according to budding sprouting was measured in the spring.

The views of “translocated” graft-incompatibility were examined by the visual symptoms of shoots and leaves according to Moreno *et al.* (1993). Scion length (cm), scion diameter (mm), rootstock diameter (mm), and young tree length (cm) were measured in the following dormant season for scion growth performance in budded plants. To evaluate the graft compatibility, trunk development was

measured at the graft union area, 5 cm above and 5 cm below, using a digital caliper (Caliskan & Karaman, 2018). In addition, the rootstock diameter/scion diameter ratio was used to estimate grafting incompatibility according to Reig *et al.* (2018a).

Anatomical and histological studies

To determine the compatibility between the grafting combinations consisting of different rootstocks, three biological samples (individual trees) were taken from each rootstock-scion combination, representing 1 cm below and above the grafting sites. Thus, anatomical and histological examinations were evaluated in grafting samples taken at 12 months for ‘localized’ incompatibility. The samples were preserved in 80% ethanol until sectioning. Then, 25 µm sections were cut directly with the aid of a rotary microtome (SM 2000 R Leica, Germany). These sections were photographed under the light microscope (Nikon Eclipse E 600, Japan) and stereomicroscope (Leica MC 165 C, Germany) after staining with 1% Safranin-O. Callus formation, condition of necrotic layers, vascular differentiation, and formation of new vascular tissues were assessed in these samples. According to these observations, graft compatibility was classified as proposed by Mosse & Herrero (1951): A, perfect unions (the line of the union between bark and wood is hardly visible); B, good unions (the bark and wood are continuous although the line of the union in the wood is often clearly distinguished by excessive ray formation); C, unions with discontinuities in the bark (the bark tissues of rootstock and scion are separated by a dark brown layer of corky appearance); D, unions showing vascular and wood discontinuities (the woody tissues of rootstock and scion are separated in many places by clusters of living, non-lignified parenchyma); and E, observed breakage of the tree at the graft union in the nursery or orchard.

Statistical analysis

The scion budding was made in rootstocks under nursery conditions, established in a randomized block design with 10 trees per block and a total of 30 young trees per each scion-rootstock graft combination. The differences between the means were determined by the Duncan multiple comparison test ($p < 0.05$) using the JUMP software program. Pearson correlation coefficients and their levels of significance were also calculated.

Results and discussion

Grafting performance

Traditional hawthorn orchards in Türkiye are established with scions ‘Sultan’ cultivar grafted on *C. azarolus*, used as hawthorn seedlings. However, clonal rootstocks that can be adapted to different climatic and soil conditions and are suitable for high-density planting will make a significant contribution to the development of modern hawthorn cultivation.

The visual symptoms of ‘Sultan’ plants showed that Sultan/Fox 11 and Sultan/Quince A presented good graft compatibility, demonstrated by healthy shoots and leaves, normal shoot growing, and standard appearance at the graft union, similar to the control rootstock (Fig. 2). In contrast, Sultan/MM 106 and Sultan/MM 111 plants displayed a common diagnosis of ‘translocated’ incompatibility, principally based on the yellowing of the leaves, the unhealthy view of shoots, and a decrease in shoot growth. These results were consistent with peach/plum graft incompatibility (Moreno *et al.*, 1993) where typical visual symptoms of incompatibility appeared on the leaves (peach yellowing and reddening) and the growth values of the scion shoot were lower compared to compatible combinations during the first year after budding. However, initial graft success values were not correlated with the incidence of graft-incompatibility. In fact, Fox 11, Quince A, and MM 106 gave higher or similar percentages of grafting success compared to other rootstocks (Table 1). The grafting success ratios ranged between 60.6% (Control) and 69.0% (Fox 11 and Quince A). These values were slightly below 70%, which is considered an ideal grafting success. However, these results agreed with those of Caliskan & Karaman (2018), who reported that the grafting success of the hawthorn/seedling ranged between 40% and 84%, depending on the grafting method and stage. Nevertheless, in the third and fourth months after the bud sprouting, growth abnormalities in shoot vigor were already observed in MM106 and MM111 rootstocks. These results showed that the success of grafting between ‘Sultan’ hawthorn and apple, pear, and quince rootstocks was not a direct indication of graft incompatibility. In contrast, Li *et al.* (2021) reported that as

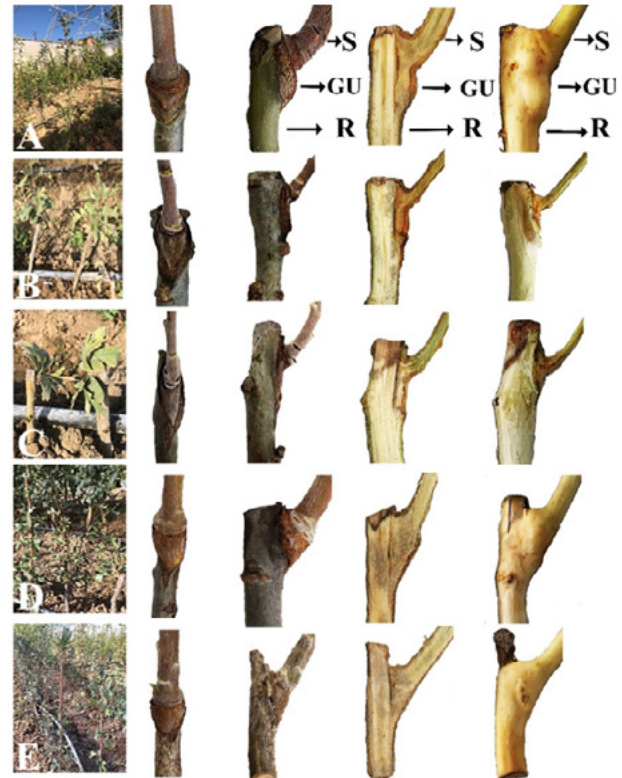


Figure 2. Budding development in the 12th-month scion/rootstock young trees for the ‘Sultan’ hawthorn cultivar on Fox11 (A), MM 106 (B), MM 111 (C), Quince A (D), and hawthorn seedling (E) rootstocks. S: scion. GU: graft union. R: rootstock.

an indicator of ‘translocated’ graft incompatibility in kiwi fruit species, the graft success decreased significantly in the days after grafting (DAG) from 99.17% to 58.47%, since 40 DAG to 80 DAG. On the other hand, in our study, the lack of continuity of the vascular tissues after grafting indicated ‘localized’ graft incompatibility, particularly in the apple rootstocks. Indeed, when morphological observations were made 12 months after budding, a healthy scion development was observed on the hawthorn seedlings, as well as on the Fox 11 and Quince A clonal rootstocks. In addition, a dense and well-structured callus tissue was formed in the graft region for these compatible rootstocks (Fig. 2). On the contrary, scion development and callus tissue were insufficient in the case of MM 106 and MM 111 apple rootstocks, especially on MM 111, where the resulting shoots reached only around 4.50 cm. In contrast, the scion cultivar exhibited the longest scion length on Fox 11 (43.18 cm) and hawthorn seedlings (31.52 cm) rootstocks. In turn, the highest scion diameter was also obtained from plants on Fox 11 and control rootstocks (7.47 mm and 6.64 mm, respectively), while the lowest scion diameter was obtained on the MM 111 (2.65 mm) and MM 106 (4.29 mm) apple rootstocks. Regarding the rootstock diameter, the highest values were shown in Quince A (11.13 mm) and Fox 11 (11.03 mm) rootstocks, whereas

Table 1. Tree vigor and grafting performance of the ‘Sultan’ hawthorn cultivar budded on different clonal rootstocks and hawthorn seedlings

Characters	Control ^[1]	Fox 11	MM1 06	MM 111	Quince A
Graft success (%)	60.6 b	69.0 a	66.7 a	62.5 b	69.0 a
Scion length (cm)	31.52 a	43.18 a	13.99 b	4.50 b	18.02 b
Scion diameter (mm)	6.64 ab	7.47 a	4.29 c	2.65 c	5.70 b
Rootstock diameter (mm)	9.20 b	11.03 a	10.06 ab	9.07 b	11.13 a
Graft union diameter (mm)	13.74 ab	15.04 a	10.60 c	7.73 c	12.75 b
Scion/rootstock ratio	0.73 a	0.67 a	0.43 bc	0.30 c	0.51 b
Rootstock/union ratio	0.69 c	0.73 c	0.95 b	1.17 a	0.88 b
Graft compatibility category ^[2]	A	A	D	D	B

^[1]Hawthorn seedlings were used as a control in the trials. ^[2]Graft compatibility category (A, B, D), according to Mosse & Herrero (1951). Means in rows followed by the same letters are not significantly different at the 5% level.

the lowest values were found in the MM 111 (9.07 mm) and the control (9.20 mm) rootstocks. In the case of graft diameter values, the highest values were measured in the control (13.74 mm) and the Fox 11 (15.04 mm) rootstocks, and the lowest values were observed in the MM 111 (7.73 mm) and MM 106 (10.60 mm) apple rootstocks (Table 1). It is well known that the growth vigor of both rootstock and cultivar used in grafting can directly affect shoot development in commercial grafting (Hartman *et al.*, 2002; Pio *et al.*, 2008). Similarly, Öztürk (2021) reported that the shoot length and diameter of the 13 pear genotypes grafted on different pear and quince rootstocks mainly depended on the rootstock vigor.

The scion/rootstock and rootstock/union diameter ratios exhibited statistically significant differences among the different scion/rootstocks graft combinations (Table 1). The highest scion diameter/rootstock diameter ratio was found in the Fox 11 pear and control rootstocks (0.73 and 0.67, respectively), whereas the lowest rootstock diameter/union diameter ratio was observed in the control and Fox 11 rootstocks (0.67 and 0.73, respectively). In addition, the

best graft combination occurred when the scion/rootstock ratio was lower than 0.7 and the rootstock/union ratio was lower than 0.8. According to Mosse & Herrero (1951), the control and Fox 11 rootstocks were classified in the category A, showing the best graft compatibility category with rootstock diameter/union diameter. Sultan/Quince A exhibited union features included in the B category. In contrast, anatomical investigation of the hawthorn ‘Sultan’ graft unions demonstrated the ‘localized’ incompatibility (category D) on MM 106 and MM 111 clonal rootstocks. Graft fusion tissues on these rootstocks had necrotic spots and no lignified tissues (Figs. 2 and 3). These results are comparable to those obtained from Reig *et al.* (2018a) who indicated that a clear ‘localized’ incompatibility (category ‘D’) could be identified between some apricot cultivars and plum rootstocks in the first year after grafting. In addition, our results showed that the scion/rootstock and rootstock/union properties could be used to predict the occurrence of graft incompatibility in hawthorn. Similarly, Reig *et al.* (2018a) reported that a value of 0.8 as the rootstock/scion ratio could be relevant in determining graft incompatibility.

Table 2. Correlation coefficients (r) of tree vigor characteristics were determined for grafting affinity tests.

Source ^[1]	SL	SD	RD	GUD	SRR	RUR	GCC
SL	1	0.96**	0.43	0.94*	0.92*	-0.90*	0.89*
SD		1	0.53	0.99**	0.95*	-0.96**	0.94**
RD			1	0.58	0.25	-0.33	0.37
GUD				1	0.93*	-0.95*	0.92*
SRR					1	-0.99**	0.94*
RUR						1	-0.90*
GCC							1

^[1]SL: scion length. SD: scion diameter. RD: rootstock diameter. GUD: graft union diameter. SRR: scion/rootstock ratio. RUR: rootstock/union ratio. GCC: graft compatibility category. * $p \leq 0.05$. ** $p \leq 0.01$.

ty. When this value was higher it could be related with the abnormalities at the graft union, probably by assimilates and water blockage. However, Ünal & Özçağiran (1986) indicated that the differences observed in the diameter of the scion/rootstock are not always associated with graft incompatibility and that this might be due to weak callus formation at the graft site and unsuitable conditions before and after grafting.

There were significant correlations between graft compatibility and shoot length and diameter, graft union diameter, scion/rootstock ratio, rootstock/union ratio, and stem diameter (Table 2). Results showed a high correlation value between the shoot diameter and graft compatibility ($r=0.94$, $p<0.01$). The shoot length, graft union diameter, and scion/rootstock ratio were found to be moderately correlated with graft compatibility. These results showed that increases in shoot length and diameter, graft union diameter, and scion/rootstock ratio values were indicators of compatible graft combinations. Besides, the rootstock/union ratio was inversely related to graft compatibility. These results are consistent with the findings of Fadel *et al.* (2019), who reported that the ‘Pera’/Crova Rangpur graft combination with a high scion/rootstock diameter ratio showed a good compatibility, while ‘Pera’/Swingle citrumelo, with a lower scion/rootstock diameter ratio, showed a poor compatibility. Similarly, another study with different peach/*Prunus* spp. graft combinations (Zarrouk *et al.*, 2006) showed a significant correlation between scion/rootstock diameter and incompatibility. Nevertheless, no significant differences were found between the scion/rootstock diameter values in apricot cultivars grafted on plum rootstocks (Reig *et al.*, 2018a; Irisarri *et al.*, 2021).

Anatomical and histological studies

The first vascular tissues formed in the callus bridge are the xylem and phloem, which repair the wound caused by budding. Wound-repairing xylem tissues mostly originate from the activity of scion tissues (Hartmann *et al.*, 2002). In our study, the slowdown in the activity of the scion tissues, the decrease in the development of the budded scion cultivar, and the persistence of the necrotic areas may be explained because the vascular connection between the graft individuals was not fully established for the apple rootstocks. Baron *et al.* (2019) reported that the failure of the graft to grow may be due to the inability to carry phytohormones, carbohydrates, and mineral substances from the grafting point or to a smaller amount of them due to the incomplete transmission between the rootstock and scion. In addition, Amri *et al.* (2021) showed that ‘translocated’ graft-incompatibility can adversely affect the transport of the non-structural carbohydrates, such as starch and other biochemical compounds, as total phenolic content and flavonoids, from the scion to the incompatible rootstock. These effects should be further tested in the MM 111 and

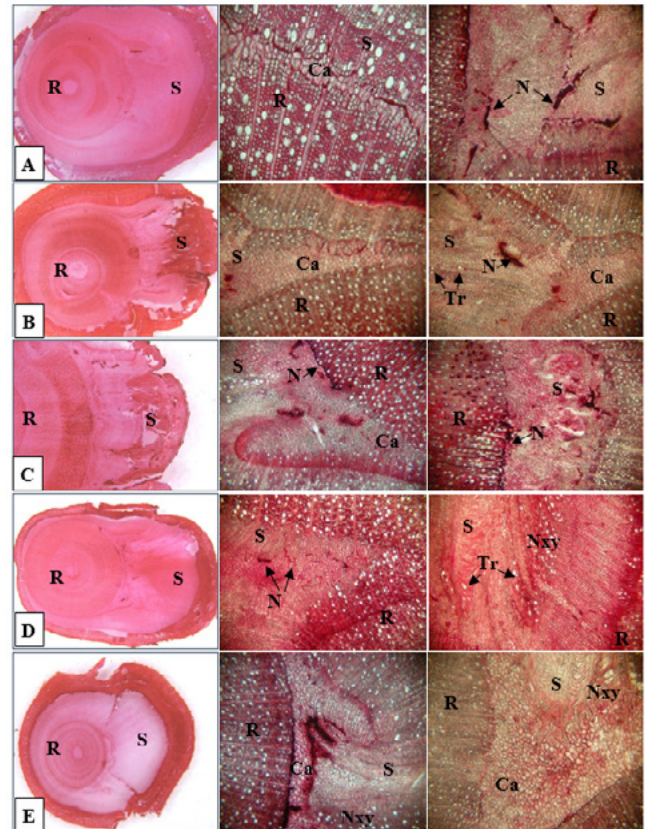


Figure 3. Budding development and situation of tissues in the 12th-month budding samples in the ‘Sultan’ hawthorn cultivar on: Fox 11 (A), MM 106 (B), MM 111 (C), quince A (D), and hawthorn seedling (E) rootstocks. S: scion. R: rootstock. GU: graft union. Ca: callus. N: necrotic layer. Tr: trachea. Nxy: new xylem.

MM 106 clonal apple rootstocks budded with hawthorn scions, aiming to deepen our knowledge on the graft-incompatibility between apple rootstocks and hawthorn.

Necrotic areas at graft interfaces and discontinuities in phloem and xylem tissues are very critical in the development of both graft association and establishment of a good vascular connection (Irisarri *et al.*, 2017). Those areas are formed because of damage to the cells on the cutting surfaces of the rootstock and scion during the preparation of the grafting. Most of these areas disappear later or remain as pockets in the callus tissue, which is formed by the active division of parenchyma cells, and in this way, the rootstock and scion are fused (Hartmann *et al.*, 2002). Similarly, Mosse (1962) reported that these necrotic layers formed by the damaged cells in the graft area were broken down by the callus tissue formed after grafting, and thus the rootstock and scion were fused. In our study, the small amount of callus layer, especially on the MM 111 rootstock, could be insufficient to break the necrotic layer formed on the graft cutting surface (Fig. 3C). In addition, sufficient fusion at the lateral junctions of all graft combinations was observed, but the presence of necrotic lines along the junction of rootstock and scion in 12-month graft

samples of MM 106 and MM 111 (Figs. 2B-C) could partially explain the weakness in scion development. Similarly, Irisarri *et al.* (2017) reported that no necrotic lines and disconnections were observed in the matched graft combination, whereas there was no continuity in the wood and bark tissues besides the necrotic line in the incompatible combinations.

Cross-section photographs for all graft combinations studied are shown in Fig. 3. In graft samples, callus tissue was formed in the lateral air pockets of the grafts and between the cut surfaces. Similar to these results, Hartmann *et al.* (2002) reported that callus tissue mostly consisted of rootstock xylem. Indeed, the callus tissue at the lateral junctions consisted only of rootstock xylem. The fact that the rootstock has the root system and thus the nutrient reserves in roots and stem may explain the important contribution of the rootstock in the formation of callus tissue. While callus tissue fills well the rootstock and scion interfaces in Fox 11, Quince A, and hawthorn seedlings rootstocks, the density of callus tissue in MM 106 and MM 111 rootstocks was insufficient (Figs. 2, 3). Rasool *et al.* (2020) stated that the callus tissue is stimulated by reactive oxygen species, stimulation of genes providing resistance to stress, synthesis of enzymes, and other chemicals in response to the wound formed on graft cutting surfaces. Wounded tissues could activate genes that stimulate cytokinins that trigger callus formation. However, callus formation may be hampered by a reduction in auxin transmission from the scion to the rootstock due to the failure to establish the vascular connection in the later phases of grafting. This might be associated with insufficient callus formation in the 12-month grafted trees of MM111 and MM106 rootstocks. Mudge *et al.* (2009) reported that the intra- or inter-familial grafts are rarely compatible or unsuccessful, while grafts between different species belonging to the same genus can be compatible in many cases (Reig *et al.*, 2018a, 2019). Thus, hawthorn could be closely related to pear and genetically much more distant from apple, therefore resulting in graft incompatibility with apple rootstocks (Zarei *et al.*, 2017).

As previously mentioned, the development of a compatible graft consists of three important stages: the fusion of rootstock and scion, the formation of the callus bridge at the graft interface, and vascular differentiation (Moore, 1984). In the present study, a callus bridge was successfully formed following the union of rootstock and scion in each graft combination. Later, a good vascular connection was also well established between rootstock and scion, providing cambial continuity in Fox 11 and Quince A clonal rootstocks, and hawthorn seedlings. There was also a partial cambial continuity in the case of MM 111 and MM 106 apple rootstocks. However, the differentiation into the vascular tissues for these two rootstocks was very low; therefore, scions did not grow, especially in the MM 111 rootstock.

In this study, the graft combinations with apple rootstocks (Sultan/MM 111 and Sultan/MM 106) showed that

the two types of graft incompatibility can occur together, similar to previous reports in different fruit tree species (Moreno *et al.*, 1995; Zarrouk *et al.*, 2006). This may support that “localized” incompatibility in hawthorn/apple graft combinations might be the result of physiological anomalies in the graft fusion combination triggered by “translocated” incompatibility. These results agree with those of Zarrouk *et al.* (2006), who reported that carbohydrate blockage above the graft area in the ‘translocated’ incompatible grafts can disallow cambium formation at the graft area and so prevent vascular tissue improving and can cause the development of incompatibility in the graft combinations. Similarly, Machado *et al.* (2016) stated that there was both ‘located’ and ‘translocated’ incompatibility, between the ‘Williams’ pear cultivar and the Quince C rootstock, due to the discontinuity of the vascular tissues at the graft interface.

In conclusion, although genetic similarity is one of the most important factors in the establishment of compatible graft combinations, genetically related species do not always induce successful graft combinations. To our knowledge, several apple, pear, and quince clonal rootstocks have been analyzed for the first time in this study, using interspecific budding for hawthorn clonal propagation. Findings showed that Fox 11 and Quince A rootstocks could be initially used as clonal rootstocks for hawthorn, showing an adequate development of scion and vascular tissues at the graft union during the first year after budding. In contrast, ‘translocated’ and ‘localized’ graft incompatibility was observed in Sultan/MM 106 and Sultan/MM111 graft combinations meaning that MM 111 and MM 106 apple rootstocks are not suitable as clonal rootstocks for hawthorn trees due to the graft incompatibility incidence. Further work should be done to investigate for a longer period if delayed incompatibility could appear in the pear and quince compatible rootstocks and to determine the effect of these initially compatible rootstocks on yield, fruit quality, and other agronomic traits in hawthorn trees growing in orchard conditions.

Authors’ contributions

Conceptualization: O. Caliskan, E. Kacal.

Data curation: E. Kacal, G. Ozturk.

Formal analysis: I. Gur, H. Kocal, O. F. Karamursel, D. Kilic.

Funding acquisition: O. Caliskan, E. Kacal.

Investigation: E. Kacal, G. Ozturk

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