Journal of the American Pomological Society 65(4): 185-191 2011

Formation of Graft Union in Pear: A Histological Study by Using Light Microscopy

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Abstract

This research was carried out at the Experimental Farm of the University of Agricultural Sciences and Veterinary Medicine Iasi, Romania during 2008-2010. The anatomical structure of the graft union in heterograft combinations of four pear variety scions on quince rootstock was studied. Tissue samples were taken from graft unions six to twelve months after grafting and first fixed in ethyl alcohol then in formalin/glacial acetic acid solution. Observations were made of the callus tissue between rootstock and scion, the necrotic layers that were formed, the formation of new cambium tissue between rootstock and scion, cambial continuity and the formation of new vascular tissues in the cambium. New cambium, xylem and phloem tissues were formed early in the region of the graft union but a longer time was needed for continuous connections to form within the cambium. Abnormalities were seen within the area of the graft union with some of the scion/rootstock combinations indicating incompatibility for these particular graft combinations. The results provide the possibility of applying an early selection method that will help to predict the future compatibility level of a specific scion/rootstock combination long before any external symptoms could be observed.

Grafting is a principal method that is used in the propagation of many species of fruit trees. For pear culture, vegetatively propagated rootstocks are more frequently used than are seedling rootstocks. Quince rootstocks are widely used in Europe (6) and in Romania 80% of pear planting material is grafted onto quince. Quince compared with seedling rootstocks, reduces tree vigour and shortens the time to fruiting of the grafted tree (13). However, there are cases when pear/quince graft combinations can develop a localized type of incompatibility that has been described by a number of authors (2, 8, 10). Several researchers have attempted to investigate the reasons for this incompatability by observing the anatomical and histological structures of the tissues at the graft union (12, 16, 18). Such analyses show vascular discontinuity associated with the presence of necrotic cells in both the wood and the bark, inclusions of non-lignified cells, and invaginations or breaks within the cambium. All of these structural deficiencies cause mechanical weakness of the graft union that can lead to tree breakage at any time, sometimes years, after grafting.

Cristoferi and Santucci (4) found that the typical symptoms of localized graft incompatibility, cell necrosis and xylem discontinuity, can be observed some years after grafting. Working on the same combination of pear scion and quince rootstock, others have highlighted the differences that can occur between compatible and incompatible graft combinations in the first few weeks after grafting (2, 5, 6).

The present study was carried out to investigate the anatomical and histological development of the graft union, following chip budding, of six pear cultivars using quince as the rootstock. The research was focused on a histological study of the graft interface in an attempt to define the causes of localized graft incompatibility. An improved understanding of the phenomenon associated with incompatibility could be a useful tool in the early diagnosis of the new scion/rootstock combinations (6). The histological aspects of the new Romanian cultivars used in this research ('Euras', 'Triumf' and 'Trivale') were evaluated for first time.

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Material and Methods

Plant material. Six pear (*Pyrus communis L.*) cultivars with different levels of previously determined compatibility were investigated following grafting onto BN 70 quince (*Cydonia oblonga* Mill) rootstock: including two compatible cultivars, 'Curé' and 'Euras'; one with poor compatibility, 'Countess of Paris', and three incompatible cultivars 'Williams', 'Trivale' and 'Triumf'. Trees were budded in August using the chip budding technique. Grafting success ranged between 40 and 95% depending on the graft combination. The observations involved 50 grafts per combination and included apparently successful, partial successful and incompatible unions.

Sample preparation. Samples consisted of 5-6 cm stem segments, fixed in 70% ethyl alcohol, taken from three different parts of the grafted area: above and under the grafted area and from the joining area (Fig. 1). Samples were taken six to twelve months after chip budding. The sections were made using a microtome (CUT 6062 Slee Mainz), and both transverse and longitudinal sections were taken through the grafted area. Section thickness varied between 15 – 22 μm.

Sections were fixed in 1% formalin-glacial acetic acid for 20 minutes and then stained using 1% methylene blue solution. The solvent was distilled water. After staining for 20 minutes, sections were well washed with distilled water and embedded in gelatine glycerinate. Sections were examined under a Motic microscope.

Microscopic observations. Samples from all six combinations, taken from three different zones (Fig. 1), above the graft area (scion), from within the graft region, and under the graft area (rootstock), were examined.

The following were defined: the formation of new vascular tissues; vessel orientation, vessel frequency in a transverse section in comparison with other anatomical elements, and vessel number per mm^2; the presence of areas containing disrupted cells; the presence of xylem rays, their width and continuity or discontinuity; the size of histological elements

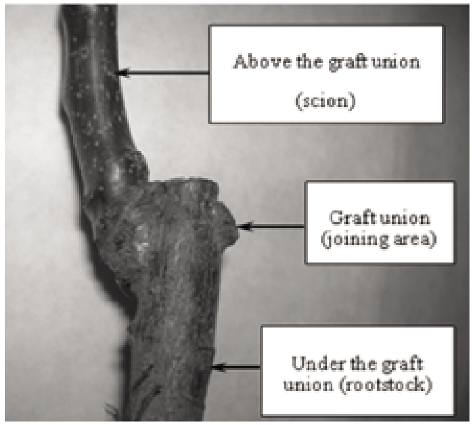

Fig. 1. The locations of the grafting area where the micro sections were taken.

(determined as the average diameter of xylem vessels); the presence of necrotic layers; the formation of new cambium tissue between rootstock and scion; and cambial continuity.

Statistical analysis. Statistical analysis was carried out using a multi-factorial analysis of variance and limit differences in order to evaluate the main sources of variance between samples and how they related to the different histological variables. Since the variants used have different levels of compatibility with the rootstock, the control was considered the variants' average.

For determination of the anatomical resemblance index, measurements of xylem vessel number and diameter were made on sections taken from each of the scion-rootstock combinations that were studied. The index was calculated using the formula defined by the Research and Development Institute for Fruit Growing, Piteşti-Mărăcineni (11), where values close to unity express good compatibility between the scion and rootstock, whereas values is greater or less than unity indicate poor compatibility.

Results

Xylem vessels with different sizes could be readily detected within the samples from above, within and below the grafted region

Table 1. Xylem vessels diameter in the graft region of six pear cultivars grafted onto quince rootstock

Cultivar	Vessel diameter in the rootstock under in the graft union (µm)	Vessel diameter within the graft union (µm)	Vessel diameter in the scion above the graft union (µm)	Graft compatibility
Curé	31.2	32.3	33.7^{xx}	Compatible
Euras	29.7	30.1	35.8^{xxx}	Compatible
Countess of Paris	22.8	26.4^{00}	30.2	Poorly compatible
Triumf	21.8	34.1^{x}	27.7	Incompatible
Williams	23.1	31.9	25.6^{00}	Incompatible
Trivale	24.4	33.8	22.9^{000}	Incompatible
Mean	25.5	31.4	29.3	
	LD 5% = 10.3	LD 5% = 2.7	LD 5% = 1.9	
	LD 1% = 15.6	LD 1% = 4.1	LD 1% = 3.0	
	LD 0.1% = 25.0	LD 0.1% = 6.5	LD 1% = 4.8	

LD limit differences compared to the mean

$^{xxx, xxx, x}$ very significant, distinctly significant, and significant, respectively, for positive differences to the control

$^{ooo, oo, o}$ very significant, distinctly significant, and significant, for negative differences to the control

(Table 1). Vessels from the area of the graft union with incompatible combinations were wider in diameter compared with those in the adjacent scion and rootstock tissues. The number of vessels from both the graft zone and the rootstock was significantly reduced, especially in incompatable combinations, compared with the values registered from the scion tissues (Table 2). Cambial discontinuity was clear, especially in incompatable combinations, and it influenced the formation of new xylem and phloem with big differences occurring between the three tissue regions studied.

The data obtained for vessel number and diameter allowed the anatomical resemblance index to be calculated and related to cultivar compatibility level. An index close to unity and, therefore, good compatibility was associated with the cultivars 'Curé' and 'Euras' (1.03 and 0.96, respectively). In contrast, for

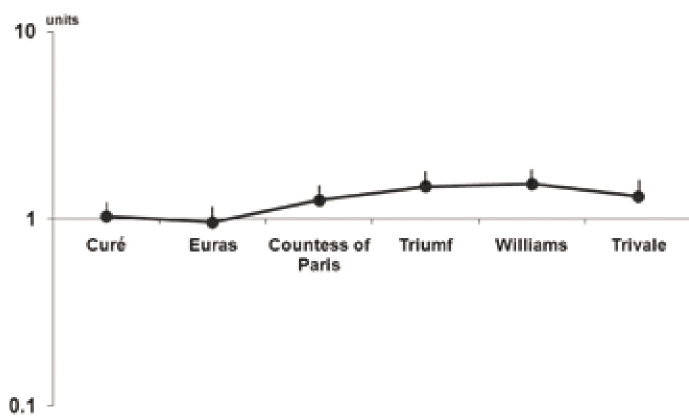

Fig. 2. The anatomical resemblance index of six pear cultivars grafted onto the rootstock BN 70 *(Cydonia oblonga)*. An index value close to unity indicates good compatibility (such as 1.03 for 'Curé' and 096 for 'Euras'), while an index which deviates from unity (such as 'Countess of Paris' (1.26), 'Triumf' (1.49), 'Williams' (1.54) and 'Trivale' (1.32)) indicates poor compatibility.

Table 2. Xylem vessel number per mm^2 in the graft region of six pear cultivars grafted onto quince rootstock

Cultivar	Vessel number per mm^2 in the rootstock under the graft union	Vessel number per mm^2 in the graft union	Vessel number per mm^2 in the scion above the graft union	Graft compatibility
Curé	55.8^{xxx}	53.1^{xxx}	62.3^{xx}	Compatible
Euras	54.6^{xxx}	54.4^{xxx}	59.6^{x}	Compatible
Countess of Paris	25.4^{000}	33.5^{000}	49.7^{0}	Poorly compatible
Triumf	27.1^{000}	36.4^{00}	50.9	Incompatible
Williams	28.3^{000}	38.6	54.2	Incompatible
Trivale	26.2^{000}	35.7^{00}	52.8	Incompatible
Mean	45.5	41.6	54.9	
	LD 5% = 3.7	LD 5% = 3.3	LD 5% = 4.4	
	LD 1% = 5.6	LD 1% = 5.0	LD 1% = 6.6	
	LD 0.1% = 8.9	LD 0.1% = 8.1	LD 1%=10.6	

LD limit differences compared to the mean
$^{xxx, xxx, x}$ very significant, distinctly significant, and significant, respectively, for positive differences to the control
$^{ooo, oo, o}$ very significant, distinctly significant, and significant, for negative differences to the control

'Countess of Paris', 'Triumf', 'Trivale' and 'Williams' the value of this index varied between 1.26 and 1.54, indicating a strong lack of compatibility (Fig. 2).

The results also revealed a direct relationship between other scion-rootstock anatomical features and graft compatibility. In incompatible combinations, there were often small necrotic areas in the wood vessels and the accumulation of large amounts of phenolic compounds in xylem rays. There were also often growth defects, observed as large areas of disrupted cells and vessels with a non-linear orientation (Figs. 3 and 4). Vessels with oblique orientation in transverse section were more frequent in incompatible combinations than in compatible combination where the vessels are properly aligned and not interrupted. We also observed large variations in the widths of xylem rays above and under the graft area with incompatible combinations.

Trees showing these anatomical features in the grafting zone can often appear, however, to be well developed. This maybe because rootstock vessels can pass to the scion through callus tissue and even through areas made up of undifferentiated cells. Nonetheless, the physical union in such situations is usually very weak (Fig. 5).

Fig. 3. Longitudinal section through the graft union of the incompatible cultivar 'Williams'. The arrows show fibres and vessels with oblique orientation and the stars indicate large disruptions to the cambium. Bar = 0.1 mm.

Localized graft incompatibility was associated in most cases with tissue necrosis, the

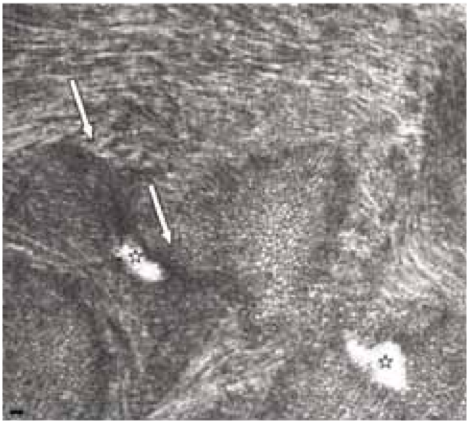

Fig. 4. Transverse section through the graft union of the incompatible cultivar 'Williams'. Arrows indicate the clear separation line between scion and rootstock. The stars indicate the presence of disrupted areas that contain non-differentiated cells. Bar = 0.1 mm.

typical symptom of incompatibility, but this was present only in samples where the graft was older than six months. This confirms other results indicating that the necrosis does not appear in the early stages following grafting (5). This necrotic layer persisted long after grafting and the formation of new vascular elements did not remove the necrosis at the interface of the scion and rootstock. In transverse section, many thin vessels in the graft zone and a necrotic layer between the old and new xylem of the rootstock were observed. With incompatible combinations, vessels showed a broad range of sizes and frequent disruptions (Fig. 6) and in some cases the new vascular elements appeared to be disoriented. With the compatible scion-rootstock combinations, vascular elements that re-established after grafting were normal and vertical alignment of the vessels occurred (Fig. 7).

Discussions

The re-establishment of vascular continuity is the last stage in the successful formation of a functional graft union and is considered by most authors to be the basic requirement for a successful graft. New vascular connections that are not well differentiated are considered to be the main reason for graft union incompatibility (7). Incompatible grafts have been reported to have reduced hydraulic conductivity in the grafted area (14).

Xylem vessel discontinuity was found in this study and, in some rootstock-scion combinations, vessels had a twisted, sinuous aspect. With all grafted plants, vessel continuity is interrupted during grafting and the recovery of conductivity is critical for the proper functioning of the new composite plant. In species and varieties with good compatability between the scion and the rootstock, the restoration of vessel continuity occurs readily and both nutrient and carbohydrate transport is almost identical to that in non grafted plants. In this regard, some authors (1, 9, 17) confirm that structural

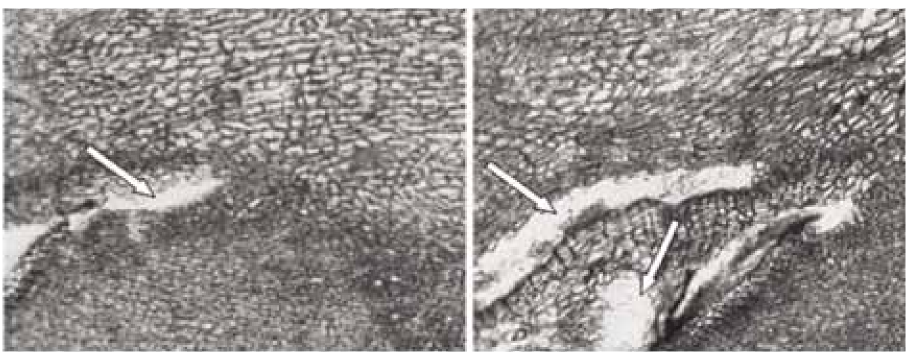

Fig. 5. Transverse section through the graft union of the incompatible cultivars 'Trivale' (left) and 'Williams' (right). Wood cells appear disorganized. Arrows indicate big disruptions that lead to poor mechanical resistance.

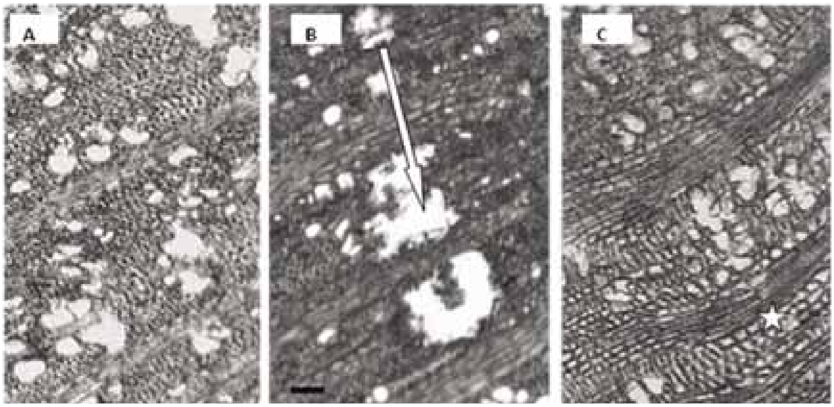

Fig. 6. Xylem vessels shown in a transverse section at different levels of graft compatibility for three pear cultivars: 'Countess of Paris' (A), 'Triumf' (B) and 'Trivale' (C). The incompatible cultivars (B, C) have vessels of different sizes and large disruptions within the xylem tissue (arrow); vessels with different orientation can also be seen (star). Bar = 0.1 mm.

abnormalities are the main cause of disturbance to the transport of photoassimilates in incompatible rootstock-scion combinations.

Vessel diameter was greater in samples taken from the scion regions than those taken from the rootstock regions, especially in compatible graft unions. In incompatible combinations, therefore, transport of photoassimilates through the graft union is restricted and delayed. Hence, a large amount of photoassimilates can accumulate above the graft union and not be transported to the roots. In such circumstances, high concentrations of sucrose and starch can be detected above the graft union (15), leading to big differences in the tree's diameter above and below the grafted area. These differences are often the first symptoms that indicate incompatibility between scion and rootstock.

A brown layer between the scion and the rootstock is due to the accumulation of toxic phenolic substances in the area of the graft union (7), which will eventually cause tissue death.

Macroscopic observations made on similar trees to those used in this study correlated well with microscopic observations, especially those taken from transversal sections through the graft union area. These observations revealed the presence of anatomical and morphological incompatibility symptoms in those scion-rootstock combinations that were known to be incompatable. These symptoms were associated with poor tree growth after grafting, the termination of vegetative growth, low success in the percentage of successful grafts, differences in the subsequent diameter between the scion and rootstock, and hypertrophy of tissues in the area of the graft. These characteristics were also related to values determined from the anatomical resemblance index that deviated strongly from unity and to weak mechanical resistance in the grafting area (3).

In conclusion, this research showed that incompatible rootstock-scion combinations had poor structural union, vascular tissue disruptions and a high proportion of necrosis in regions in the graft zone. Further, the callus that formed did not completely fill the spaces between the scion and the rootstock and, as result, the presence of non differentiated cells were a typical symptom of incompatible combinations. The presence of a clear separation line, of cell necrosis and of large lacunar areas were main indicators of graft incompatibility. An improved understanding at the histological level provides the possibility of an early diagnosis of incompatability in new scion/rootstock combinations.

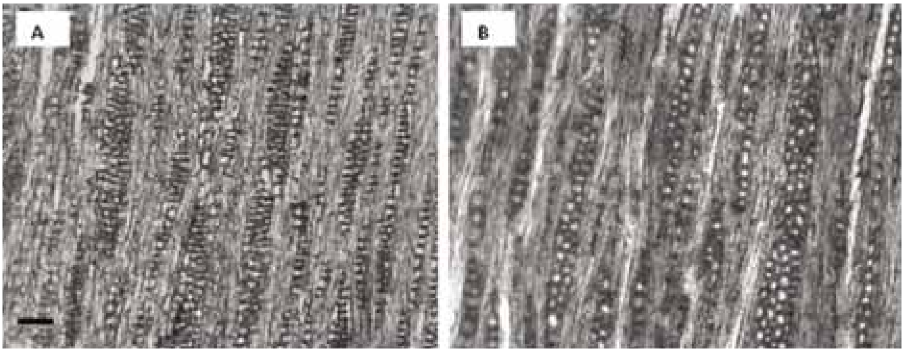

Fig. 7. Normal arrangement of vessels in tangential longitudinal section for two compatible cultivars 'Curé' (A) and 'Euras' (B). Bar = 0.1 mm.

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