# INVESTIGATION OF ANATOMICAL STRUCTURE OF GRAFT UNION IN SWEET CHERRY

## INVESTIGAȚII ASUPRA STRUCTURII ANATOMICE A ZONEI DE ALTOIRE LA CIREŞ

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**Abstract:** *The research was aimed to determine the anatomical structure of graft union in some combination of sweet cherry. The study was carried out in the University of Agricultural Sciences and Veterinary Medicine experimental farm during 2007-2008. There were used four scion varieties (Germersdorf, Stella, Van and Boambe de Cotnari), two different rootstocks (Cerasus avium seedling and mahaleb). T budding grafting method was used. Tissue samples were taken twelve months after grafting and fixed in alcohol solution. It was observed from the anatomical structure of graft union area that new cambium, xylem and phloem tissues were formed and there was needed longer time for continuous cambial merging. Some abnormalities were seen at graft union area at some combinations and it was suggested that there could be a not very good compatibility for these graft combinations.*

**Key words:** grafting compatibility, sweet cherry, anatomical structure

***Rezumat:*** *Scopul principal al lucrării a fost determinarea structurii anatomice a zonei de altoire la unele soiuri de cireş. Studiul s-a desfăşurat în ferma experimentală a Universității de Ştiințe Agricole şi Medicină Veterinară Iaşi, în perioada 2007-2008. S-au folosit patru soiuri de cireş (Germersdorf, Stella, Van şi Boambe de Cotnari), altoite pe doi portaltoi: cireş franc şi mahaleb, sa metodă de altoire folosindu-se altoirea în T cu mugure dormind. Probele de țesuturi s-au prelevat după 12 luni de la altoire şi au fost fixate în soluție alcoolică. Din observațiile privind stuctura anatomică a zonei de altoire s-a evidențiat faptul că noile elemente vasculare s-au format într-o anumită proporție şi că a fost nevoie de mai mult timp pentru restabilirea continuității vasculare. S-au observat unele anomalii în structura zonei de altoire, ceea ce a condus la concluzia că soiurile au un anumit grad de incompatibilitate cu portaltoiul mahaleb.*

**Cuvinte cheie:** compatibilitate la altoire, cireş, structură anatomică

## INTRODUCTION

Grafted plants are intensely used in fruit production and this is why choosing the best association scion-rootstock is vital. Knowing the changes that occurs at the grafting area makes us understand better the incompatibility mechanism and allowing us to engraft a larger number of varieties on a compatible rootstock.

Researches have long assumed that graft compatibility-incompatibility in plants is determined by a cellular recognition system [8,9]. However this assumption has been made without convincing data, and has been based primarily on evidence implicating cellular recognition in other compatibility responses in plants. The aim of this paper was to determine the anatomical structure of graft union in some combination of sweet cherry and find the implications that could

explain graft compatibility-incompatibility between some varieties and rootstocks. It was found that vascular redifferentioation is the final event that typically occurs in the formation of a compatible graft. At the analyzed combinations, from the anatomical structure of graft union area, was observed that new cambium, xylem and phloem tissues were formed and there was needed longer time for continuous cambial merging. Some abnormalities were seen at graft union area at some combinations and it was suggested that there could be a not very good compatibility for these graft combinations.

The applicability of this study could be the possibility to apply an early selection method that could predict the future of a determinate combination long before the external symptoms can be observed.

## MATERIAL AND METHOD

***Anatomo-morphologic observations*** were made using fresh material, taken during the vegetation period (July-August 2008). The probes consisted of 3-4 cm stem fragments of the grafting area, which were fixed afterwards in ethylic alcohol 70$^{o}$.

In order to diagnostic some aspects of grafting incompatibility there were taken samples from grafted combinations. The sections were made using microtome CUT 6062 Slee Mainz, and there were performed transversal and longitudinal sections through the joining area. Sections thickness varied between 15 – 22µ. The sections were fixed in glacial acetic acid 1% time for 20 minutes. After colouring in metilene blue solution for 20 minutes, probes were washed with distillate water and included in gelatine glycerinate. The probes we obtained were analyzed at Motic microscope with size unit 10x18 and objectives 4/0.10 and 10/0.25.

On micro sections there were made observations concerning: xylem vessels orientation; vessels way of arrangement, if they are linear or if they present involution and sinuous aspect, if xylem fascicle is continuous or if it's interrupted in the joining area; vessels frequency determination in transversal section in comparison with other anatomical elements (the was also determined the number of vessels in the grafting area); the presence of lacunars area; medullar rays width; medullar rays continuity or discontinuity; determination of histological elements size (average diameter of xylem vessels); determination of simbionts anatomical resemblance index (I.C.D.P. Piteşti-Mărăcineni).

**For determination of simbionts anatomical resemblance index** there were made measurements of xylem vessels number and diameter on sections took from combinations scion-rootstock that was studied. With the obtained data there was calculated the simbionts anatomical resemblance index using the formula elaborated by I.C.D.P. Piteşti-Mărăcineni [11]:

$$\textbf{I. a. at. s.} = \frac{\dfrac{Nr.v.a./Nr.v.p. + Nr.v.a/Nr.v.p.alt. + Nr.v.p./Nr.v.p.alt.}{3}}{\dfrac{\phi v.a./\phi v.p. + \phi v.a/\phi v.p.alt. + \phi v.p. + \phi v.p./\phi v.p.alt}{3}}$$

where: I. a. at. s. = simbionts anatomical resemblance index;
Nr. v. a. = xylem vessels number at 10 cm above the joining area;
Nr. v. p. = xylem vessels number at 10 cm under the joining area;
Nr. v. p.alt. = xylem vessels number in the joining area;
Φ v. a. = vessels average diameter at 10 cm above the joining area
Φ v. p. = vessels average diameter at 10 cm under the joining area;
Φ v. p.alt. = vessels average diameter in the joining area.

## RESULTS AND DISCUSSIONS

At sweet cherry varieties the most obvious symptoms of incompatibility were visible differences between scion and rootstock diameter. Grafting success percentage was high so, it can not be correlated with the presence of scion and rootstock's tissues hypertrophies in the grafting area. Analyzing the sections at sweet cherry/mahaleb associations there could be observed that tissues hypertrophy, most of the times, was not associated with serious abnormalities of the vessels. Data from literature presents situations when vessels normal trajectory can occur even years after grafting [7].

In figure 2, which represent transversal section through the grafting area, can be observed xylem vessels and in longitudinal section, from photo 1, there could be observed some areas with vessels discontinuity but on some areas vessels continuity was re-established, fact that drove to trees normal development in the first years after grafting. Also, there could be observed some necrotic spots and areas with undifferentiated parenchyma with small and irregular cells, which form a fragile structure weakening trees resistance in the grafting area.

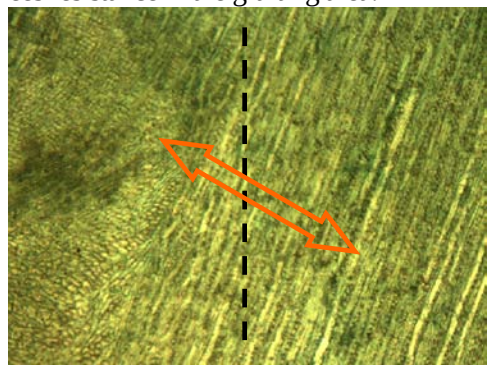

**Fig. 1.** Longitudinal section through joining area, can be observed vessels continuity (right) and areas with undifferentiated parenchyma and necrotic spots (left), Germersdorf variety

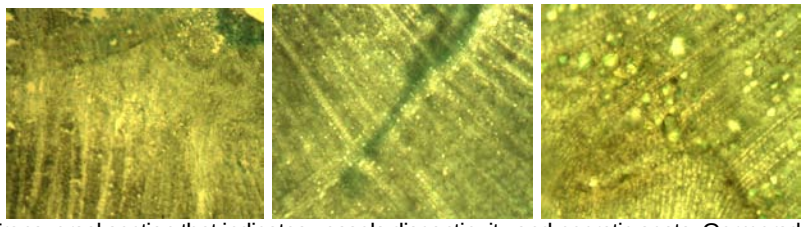

**Fig. 2.** Transversal section that indicates vessels discontinuity and necrotic spots, Germersdorf variety

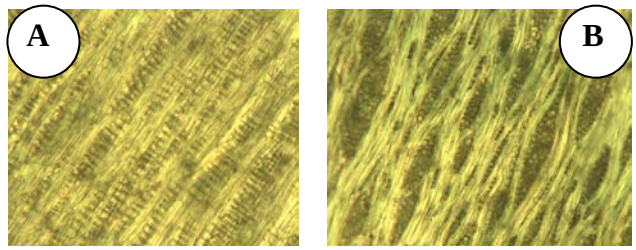


**Fig. 3.** Normal trajectory of the vessels in the joining area, longitudinal section, A-Stella, B-Van

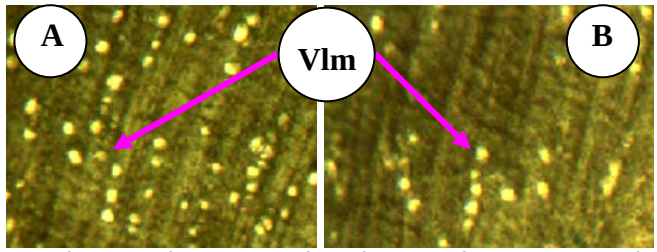


**Fig. 4.** Xylem vassels aspect in transversal section: A- scion, B – rootstock - Stella variety

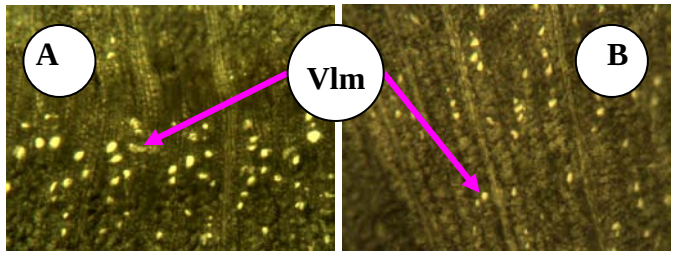


**Fig. 5.** Xylem vassels aspect in transversal section: A-scion, B-rootstock; - Boambe de Cotnari variety

In figure 3 at heterograft combinations Van/mahaleb and Stella/mahaleb we can see the parallelism and normal aspect of the vessels in the joining area, while in photo 6 are obvious the differences in vessels number determined above and under the grafting area at Boambe de Cotnari variety.

Data concerning sweet cherry varieties level of compatibility drove us to a series of anatomical resemblance index that allowed us to appreciate the varieties compatibility scale with the rootstock. This showed a good compatibility of the varieties grafted on mahaleb, limits of variation of this index varying between 0.98-1.18. Van and Stella varieties had a better compatibility, expressed by value of the index very close to the unit (0.98 respectively 1.01), (fig. 6.).

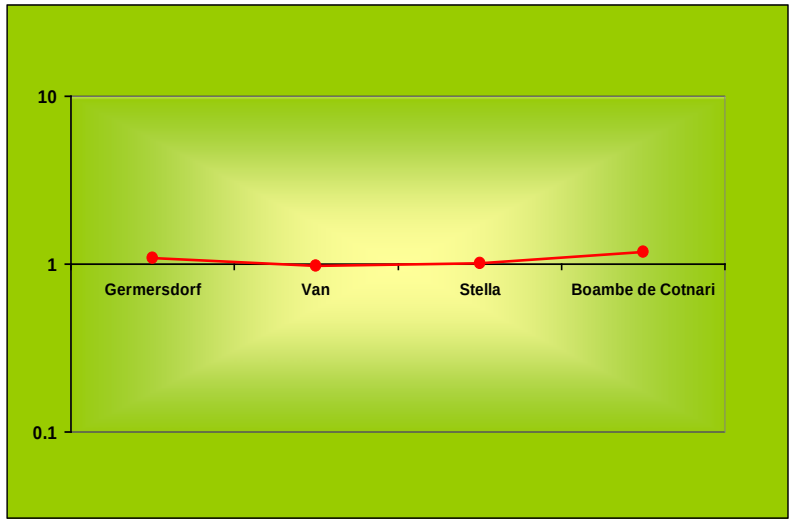


**Fig. 6.** Simbionts anatomical resemblance index at sweet cherry varieties

*Table 1*

**Statistical assurance of xylem vessels number in three analyzed areas at sweet cherry varieties grafted on mahaleb**

| Variety/Rootstock | Vessels nr. under the grafting area | Difference to the average ± | Signif. | Vessels nr. in the joining area | Difference to the average ± | Signif. | Vessels nr. above the grafting area | Differenc e to the average ± | Signif. |
|---|---|---|---|---|---|---|---|---|---|
| **Germersdorf** | 46.7 | -2.3 | 0 | 42.8 | -0.7 | - | 54.3 | +2.3 | - |
| **Van** | 47.9 | -1.1 | - | 47.2 | +3.7 | 00 | 55.8 | +3.8 | - |
| **Stella** | 42.8 | -6.2 | 000 | 38.8 | -4.7 | 00 | 39.1 | -12.9 | 000 |
| **Boambe de Cotnari** | 58.5 | -9.5 | 000 | 45.2 | +1.7 | - | 57.5 | +5.5 | x |
| Average | 49.0 | - | - | 43.5 | - | - | 52.0 | - | - |
| | DL 5% = 2.30<br>DL 1% = 3.49<br>DL 0.1% = 5.60 vessels | | | DL 5% = 2.06<br>DL 1% = 3.12<br>DL 0.1% = 5,01 vessels | | | DL 5% = 3.74<br>DL 1% = 5.67<br>DL 0.1% = 9.11 vessels | | |

*Table 2*

**Statistical assurance of xylem vessels number in three analyzed areas at sweet cherry varieties grafted on mahaleb**

| Variety/Rootstock | Vessels diameter under the grafting area | Difference to the average (μ) | Signif. | Vessels diameter in the joining area | Difference to the average (μ) | Signif. | Vessels diameter above the grafting area | Difference to the average (μ) | Signif. |
|---|---|---|---|---|---|---|---|---|---|
| **Germersdorf** | 3.06 | -0.04 | - | 2.88 | -0.09 | - | 3.18 | -0.01 | - |
| **Van** | 3.01 | -0.09 | - | 2.89 | -0.08 | - | 3.46 | +0.27 | - |
| **Stella** | 2.99 | -0.11 | - | 3.01 | +0.04 | - | 2.98 | -0.21 | - |
| **Boambe de Cotnari** | 3.32 | +0.22 | - | 3.11 | +0.14 | - | 3.15 | -0.04 | - |
| Average | 3.10 | - | - | 2.97 | - | - | 3.19 | - | - |
| | DL 5% = 0.61<br>DL 1% = 0.92<br>DL 0.1% = 1.49 μ | | | DL 5% = 0.24<br>DL 1% = 0.37<br>DL 0.1% = 0.59 μ | | | DL 5% = 0.85<br>DL 1% = 1.30<br>DL 0.1% = 2.08 μ | | |

From tables 1 and 2 could be observed that a high number of vessels were determined at Boambe de Cotnari variety (58.5 vessels above the joining area and 57.5 under the joining area) and less vessels at Stella variety (42.8 vessels above the joining area and 39.1 under the joining area). In the joining area of the scion with the rootstock the number of vessels decreased at all four variants but there were big variations at Boambe de Cotnari and Stella. Van and Germersdorf varieties registered less variation in vessels number in the joining area (figures 4 and 5). These negative differences may explain xylem vessels discontinuity between rootstock and scion. Vessels diameter in the grafting area was bigger at Boambe de Cotnari variety (3.11 μ) and smaller at Germersdorf and Van varieties (2.88 and respectively 2.89 μ), but the differences determined in those three analyzed areas were insignificative.

## CONCLUSIONS

1. Our results confirm that grafting includes the formation of necrotic layer and its subsequent reduction or elimination. There were observed necrotic spots, but on some parts there was re-established vessels continuity in some proportion, fact that allowed assimilated satisfactory transport al and thought, trees normal development and growth in first years after grafting.

2. The incompatible heterografts showed a marked delay in new vascular elements formation explained by the differences in diameter and number determined above and under the joining area.

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