FORMATION OF GRAFT UNION IN PLUM: A HISTOLOGICAL STUDY BY USING LIGHT MICROSCOPY

INVESTIGAȚII ALE ZONEI DE ALTOIRE LA PRUN: STUDIU HISTOLOGIC FOLOSIND MICROSCOPIA OPTICĂ

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Abstract: *During grafting, phenolic compounds from the cut surface cells oxidize and produce necrotic layer isolating the surfaces. Callus cells formed from the xylem medullar ray and secondary shell cells destroy the necrotic layers on the cut surfaces. Then, the cavity between the rootstock and scion is filled and reestablish the connection between them. After this stage, the transport of water and nutrients through the grafting area occurs* (Buttner, 1979). *For a successful grafting it is important to pursue the anatomical development between tissue of scion and rootstock after grafting. The anatomical structure of graft unions was investigated in plum varieties 'Stanley', 'Centenar', 'Tuleu gras' and 'Pescarus' grafted on cherry plum (Prunus cerasifera Ehrh) seedlings. This research was aimed to determine the anatomical structure of graft union in some graft combinations of plum using chip-budding grafting technique. The study was carried out in 2007-2009 in University of Agricultural Sciences and Veterinary Medicine Iasi Experimental orchard. Tissue samples from graft unions were taken one year after grafting and fixed in formalin/glacial acetic acid/ethanol solution. Scattered brown necrotic layers were identified, as a result of enzymatic reactions in the junctional tissue. By analysing the pattern of the development of vascular tissues we can estimate the compatibility of the graft combination and control the grafting process* (Ermel, 1997). *The results are beneficial in nursery plant production for new rootstocks selections.*
Keywords: grafting, compatibility, plum, anatomy

Rezumat: *În timpul altoirii, compuşii fenolici produşi de celulele de la suprafaţa secţiunilor oxidează şi formează un strat necrotic izolator. Ulterior, ţesutul de calus format distruge straturile necrotice din zona de contact, iar cavitatea dintre portaltoi şi altoi se umple şi începe restabilirea legăturilor vasculare. După această etapă, transportul apei şi a nutrienţilor prin zona de altoire poate are loc în limite normale* (Buttner, 1979). *Pentru o bună prindere este important să continue dezvoltarea anatomică corespunzătoare în zona de altoire. Astfel, s-a investigat structura anatomică a zonei de altoire la soiurile de prun "Stanley", "Centenar", "Tuleu gras" şi "Pescăruş", altoite pe Prunus cerasifera Ehrh. Scopul acestor cercetări a fost determinarea structurii anatomice a zonei de altoire la combinaţiile altoi-portaltoi alese, folosind tehnica chip-budding ca metodă de altoire. Studiul a fost efectuat în câmpul experimental al Universităţii de Ştiinţe Agricole şi Medicină Veterinară Iaşi, în perioada 2008-2011. Probele de ţesut au fost prelevate din zona de altoire la un an după altoire şi fixate în formol/acid acetic glacial/soluţie de etanol. În urma observaţiilor microscopice au fost identificate straturi necrotice de culoare maro, ca urmare a unor reacţii enzimatice în ţesutul de joncţiune. Prin analiza modelului de dezvoltare a ţesuturilor vasculare se consideră că se poate estima gradul de compatibilitate a asociaţiei altoite, putând astfel controla procesul de altoire (*Ermel,

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1997). *Rezultatele îşi găsesc utilitatea în sfera producerii de material săditor pomicol şi în selecţia unor noi portaltoi.*

Cuvinte cheie: altoire, compatibilitate, prun, anatomie

INTRODUCTION

Plants produced from seeds are frequently used as roostock for plum in the production of nursery plants. In Romania, *Prunus cerasifera* and numerous clones of this specie are used in high percentage in grafting plum varieties. In this way, are occuring many differences in the development of nursery plants owing to genetic heterogenity of rootstocks. Besides the problems as overheight and long juvenile period, grafting incompatibility is another serious problem of nursery production (Errea et al., 1994). Knowing the changes that occur at the grafting area makes us understand better the incompatibility mechanism and allow us to engraft a larger number of varieties on a compatible rootstock.

In this paper there was determined the anatomical structure of graft union in some combination of plum varieties with *Prunus cerasifera* and find the implications that could explain graft compatibility-incompatibility between some varieties and rootstocks. From analyzed combinations samples we had, 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 (Dolgum et al., 2008; Tekintas et al., 1996).

The general aim of this study is the possibility to find and 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 2010). The probes consisted of 3-4 cm stem fragments of the grafting area, which were fixed afterwards in ethylic alcohol 70^{o}.

The variants were four plum varieties (Stanley, Pescăruş, Centenar and Tuleu gras) with different compatibility level at grafting on *Prunus cerasifera.*

In order to diagnostic some aspects of grafting incompatibility there were taken samples from three different parts of the grafted area: above and under the grafted area and from the joining area. 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 – 22 µ. 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) (Zlati et al., 2011).

RESULTS AND DISCUSSIONS

The macroscopic observations made after grafting at incompatible combinations highlighted differences in scion and rootstock diameter, deviations from vessels normal longitudinal orientation, weak resistance at the joining area, development of brown layer of separation between scion and rootstock by generating at the incision place of a scarring suberus (photo 1), which as is known, is formed of cells whose walls are impregnated with polyphenolic substances, which confers impermeability (Gurrieri et al., 2001).

Data on the new vascular elements formed in the grafting area at plum varieties grafted on *Prunus cerasifera* are presented in table 1 and can be noticed a higher number of vessels at those varieties who had good compatibility with the rootstock without big differences between scion and rootstock (Stanley registered 57.6 vessels above the grafting area and 54.2 vessels under the grafting area). While in incompatible varieties could be noticed big differences in vessels number above and under the grafting area (Tuleu gras registered 60.2 vessels above the grafting area and 23.9 vessels under the grafting area). These negative differences may explain vessels discontinuity between scion and rootstock and the blockage in water and nutrients transport (Cristoferi et al., 1965).

Table 1

Xylem vessels number in three analyzed areas at plum varieties grafted on *Prunus cerasifera*

Variety/ Rootstock	Vessel number per mm^2 in the rootstock under the graft union	Signif.	Vessel number per mm^2 in the graft union	Signif.	Vessel number per mm^2 in the scion above the graft union	Signif.
Stanley	54.20	xxx	44.10	-	57.60	-
Pescăruş	49.20	xx	46.80	x	54.70	-
Centenar	28.10	00	41.40	-	63.30	-
Tuleu gras	23.90	000	34.70	00	60.20	-
Control	38.85	-	41.75	-	58.95	-
	LSD 5% = 5.68 LSD 1% = 8.60 LSD 0,1% = 13.82 vessels		LSD 5% = 4.53 LSD 1% = 6.86 LSD 0,1% = 11.02 vessels		LSD 5% = 4.60 LSD 1% = 6.97 LSD 0,1% = 11.20 vessels	

Regarding vessel diameter, distinctly significant differences were recorded in the same varieties, Pescăruş and Tuleu gras (in the joining area) and Pescăruş and Centenar above the grafting area (tab. 2).

In plum, microscopic symptom of incompatibility which has been frequently observed was the presence of undifferentiated parenchyma tissues along with vascular tissues, which had a clear line of separation that prevents re-establishment of vessels

continuity in the grafting area. In some cases, in this mass of parenchymatic cells could be observed necrotic spots both in cross and longitudinal sections (photo 2).

Table 2

Xylem vessels diameter in three analyzed areas at plum varieties grafted on *Prunus cerasifera*

Variety/ Rootstock	Vessel diameter in the rootstock under the graft union (µm)	Signif.	Vessel diameter within the graft union (µm)	Signif.	Vessel diameter in the scion above the graft union (µm)	Signif.
Stanley	34.5	-	27.1	-	33.0	-
Pescăruş	30.2	-	31.2	xx	36.1	x
Centenar	36.2	-	27.8	-	29.0	00
Tuleu gras	28.6	-	22.1	00	34.2	-
Control	32.3	-	27.0	-	33.0	-
	LSD 5% = 6.10 LSD 1% = 9.20 LSD 0,1% = 14.90 µ		LSD 5% = 2.60 LSD 1% = 4.00 LSD 0,1% = 6.50 µ		LSD 5% = 2.40 LSD 1% = 3.70 LSD 0,1% = 5.90 µ	

However, the most serious symptom of incompatibility observed at plum was large lacunar areas that, in addition to the disturbances they produce in assimilate transportation, weaken trees mechanical resistance (photo 3). This symptom was manifested especially in the grafting area, but it could be also observed in the samples that were taken from the scion and rootstock tissues. Tissues defective developing at incompatible plum varieties grafted on *Prunus cerasifera* was characterized by the presence of necrotic areas, which were supposed to be the lack of callus response to stimuli for differentiation.

Another aspect observed in microscopic sections was that the new xylem vessels that were formed had a nearly horizontal orientation, turning from the normal vertical orientation (photo 5). Other researches but concerning sweet cherry varieties noticed the same aspect in heterograft combinations.

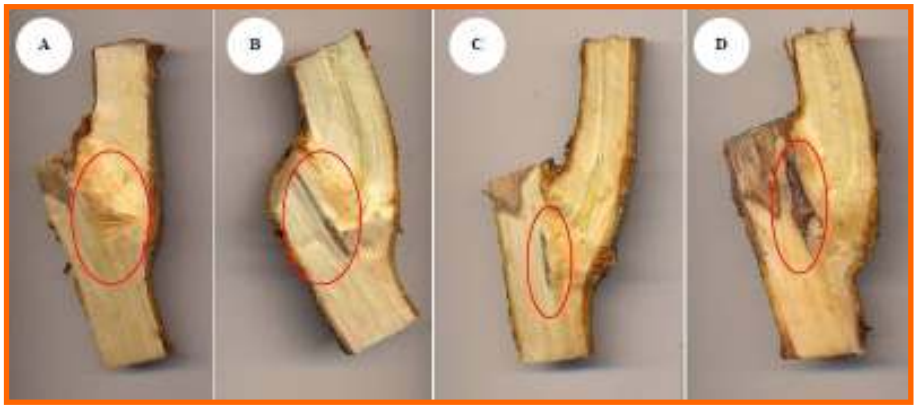

Fig. 1 - Macroscopic observations of the grafting area at plum varieties grafted *on Prunus cerasifera*: A - Stanley, B - Centenar, C - Pescăruş, D - Tuleu gras.

In photo 6 can be observed xylem vessels aspect in cross section and the big differences existent in vessels number above and under grafting area at Centenar variety (29.0 vessels above and 36.2 vessels under the grafting area) at Centenar variety. Also in some cases could be observed, in cross section, vessels discontinuity (photo 4 and 5) caused by undifferentiated parenchyma inclusions.

By comparison, there were used samples from a compatible combination, Stanley variety grafted on *Prunus cerasifera* where could be observed the normal aspect of the vessels both in longitudinal and transversal section, vessels right alignment and the absence of necrotic spots and lacunar areas.

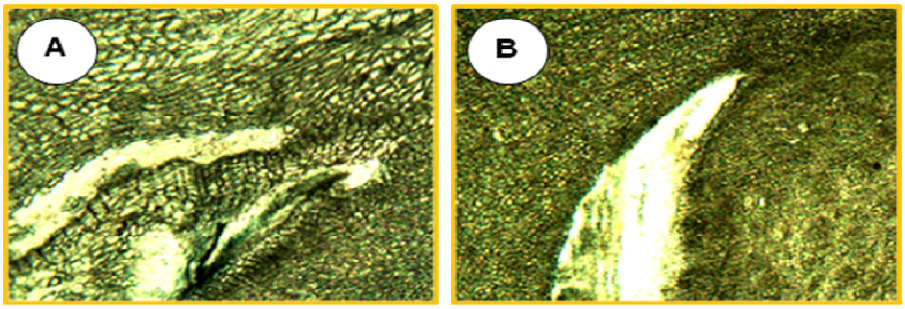

Fig. 2 - Transversal section through the joining area at Centenar variety; lacunar areas presence: A-lens 10 x, B-lens 4x

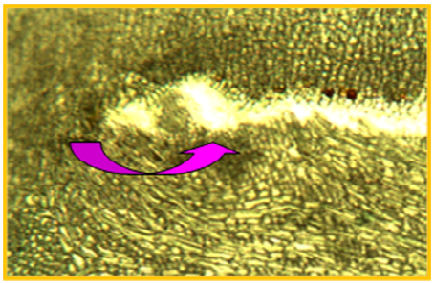

Fig. 3 - Transversal section through the joining area at Tuleu Gras variety; the presence of the lacunar areas

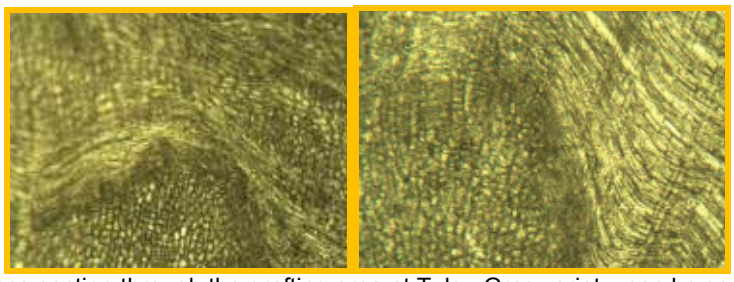

Fig. 4 - Cross section through the grafting area at Tuleu Gras variety, can be seen vessels discontinuity in the joining area

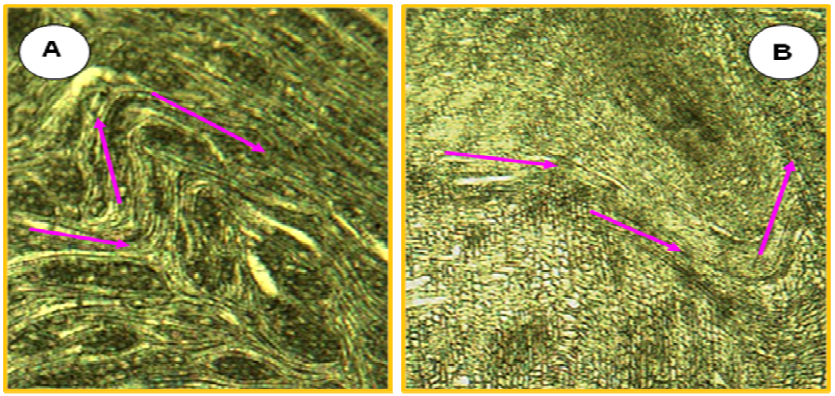

Fig.5 - Xylem vessels sinuous trajectory in the joining area at Tuleu Gras variety – longitudinal section(arrows indicate changes in vessels normal orientation): A-lens 10 x, B-lens 4x.

CONCLUSIONS

1. In compatible combinations the examined tissues were well developed, new cambium, xylem and phloem tissues were formed and there was needed longer time for continuous cambial merging.

2. 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, symptoms also observed in research we performed for other grafting combinations in pear (Zlati et al., 2011).

3. Tissues hypertrophy and large lacunar area were more obvious symptoms observed at plum varieties.

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