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Graft Incompatibility Influence on Assimilating Pigments and Soluble Sugars Amount of some Pear (*Pyrus sativa*) Cultivars

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Abstract

Graft incompatibility in fruit trees is one of the greatest obstacles in rootstocks and cultivars breeding. The mechanism in which incompatibility is expressed is not yet fully understood and several hypotheses have been advanced in an attempt to explain it. In many cases (pear on quince grafts, apricot on *Prunus* grafts), incompatibility is manifested by the breaking of the trees at the point of the union particularly when they have been growing for some years. Many reports focus on this problem in order to understand the mechanisms of graft development. These reports refer to both cytological and biochemical responses occurring at an early phase in response to grafting, as well as to the consequences of these events on the future graft response. In this experiment, we tried to highlight how affinity between scion and rootstock can influence the photosynthetic apparatus and carbohydrates synthesis. The results showed that grafting affinity has an influence on total assimilating pigments content. Thus, on the pear cultivars grafted on an incompatible rootstock (cultivars/*Cydonia oblonga*) the total pigments content ratio (reported to the ungrafted rootstock) ranged between 0.58 and 0.69. However, the combinations had a ratio ranging between 0.79 and 0.98. Nevertheless, the assimilating pigments ratio reduction had no influence on photosynthetic rate. The soluble sugars amount was close in both variants (cultivars/*Cydonia oblonga* and cultivars/*Pyrus sativa*).

Keywords: *Pyrus sativa*, *Cydonia oblonga*, carbohydrates, chlorophylls, carotenoids

Introduction

Fruit trees are usually formed by a combination of scion and rootstock. In order for that combination to be successful, a good union between the scion and rootstock is necessary (Errea *et al.*, 2001; Feucht, 1988). The grafted partners often belong to the same species or genus but the use of genetically divergent genotypes is also common. Therefore, graft incompatibility occurs frequently, especially in these inter-specific combinations. This is often the case of pear (*Pyrus sativa*) grafted on quince (*Cydonia* sp.), apricot (*Prunus armeniaca*) grafted on other *Prunus* species etc. Hartmann *et al.* (1997) named a localized graft incompatibility that which is manifested by breaking off the trees at the point of union because of anatomical abnormalities of vascular tissue in the callus bridge. This incompatibility reaction can take years to manifest, after several seasons of apparently successful growth (Fig. 4 and Fig. 5). Therefore, incompatible grafts can grow for several years without any external symptom of incompatibility indicating the presence of functional vascular connections (Errea and Felipe, 1993; Hartmann *et al.*, 1997).

For this reason, the delayed appearance of the symptoms increases the time required for detection of graft-compatibility and slows down new rootstock selection programs.

The aim of this research is to determine the effects of grafting combinations (with compatible and incompatible rootstocks) on the amount of assimilating pigments and soluble sugars in four pear cultivars. In addition, we attempt to find out whether such analysis can be a useful tool for forecasting incompatibility in breeding pear cultivars.

Materials and methods

To perform the experiment it has been used four pear cultivars. Two of them are cultivated for years on large areas in Romania ('Comtesse de Paris' and 'Williams') and other two are relatively new, autochthonous ('Trivale' and 'Triumf'). All four cultivars were grafted onto one rootstock known as incompatible (quince-*Cydonia oblonga*) and one known as compatible (*Pyrus sativa*) as a control. The experiment was conducted in the "V. Adamachi" didactic farm of the University of Agricultural Sciences and Veterinary Medicine Iasi. The analyses were conducted on four sets of nursery pear trees, one new set each year, from 2006 to 2009.

To achieve the aim of the experiment, we made two sets of tests, namely the quantitative determination of assimilating pigments in mature leaves and soluble sugars in mature leaves and from the grafting union. Both sets of tests were done twice a year, first in July and second in August, over a four year period, from 2006 to 2009. The samples were collected from mature leaves, from scions (just above the grafting union), from the union point and from the rootstock (just below the grafting union).

Assimilating pigments were extracted and determined according to the Current Protocols in Food Analytical Chemistry (Lichtenthaler and Buchmann, 2001). Fresh leaf tissues (0.1 g) were ground in acetone (pure solvent) and centrifuged at 10,000 x g for 5 minutes. Absorbance of the supernatant was read at 661.6 nm for chlorophyll *a* (Chl. *a*), 644.8 nm for chlorophyll *b* (Chl. *b*) and 470 nm for carotenoids (Car.) using a T70 UV/VIS Spectrophotometer PG Instruments Ltd.

The content of soluble sugars was determined as follows: from grafted tree leaves and stems fine grinded samples soluble sugars were extracted by hot ethanol (3 replicates). Five ml of 70% (v/v) ethanol were mixed by shaking them with 300 mg of grinded tissues in a test tube. The sample was then incubated at 80°C for 20 min. by shaking and then centrifuged for 5 min. at 1500 x g. Freshly prepared anthrone reagent (0.2% anthrone in concentrated H_2SO_4) was pipetted into a test tube and chilled in ice water. The extract was thoroughly mixed with the anthrone reagent, the tube heated in boiling water for 15 min. and then rapidly cooled. The absorbance was read at 630 nm.

Results and discussion

Quince rootstocks are widely used in Europe to grow pear cultivars. Compared with pear rootstocks, quince reduces the size and shortens the time to fruiting of the grafted tree (Browning and Watkins, 1991). However, some economically important pear cultivars are incompatible with otherwise desirable quince rootstocks. The symptoms of pear/quince incompatibility, which are typical of the localized type of graft incompatibility, have been extensively described (Herrero, 1951; Mosse, 1958). When these pear cultivars are grafted onto quince rootstocks, a cyanogenic glycoside, prunasin, which is normally found in quince but not in pear, is translocated into the pear phloem. The pear tissues break down the prunasin in the region of the graft union to give hydrocyanic acid as one of the decomposition products. Different pear cultivars vary in their content of a water-soluble inhibitor of the enzyme which catalyses the breakdown of prunasin. The presence of hydrocyanic acid at the graft union checks cambial activity there and also destroys phloem tissues at and above the union. Conduction of water and materials through the union is reduced. The reduction in the amount of sugars reaching the quince roots leads to further decomposition of prunasin, liberating hydrocyanic acid and killing quince phloem (Gur *et al.*, 1968). Therefore, the graft interface shows a vascular discontinuity associated with the presence of necrotic cells in the wood and bark, the inclusion of un-lignified cells in the wood and invagination or breaks of the cambium. The graft incompatibility also causes mechanical weakness of the union that can lead to its breakage several years after grafting. Knowledge of early structural and functional events associated with incompatibility could be used to diagnose graft compatibility of new scion/rootstock combinations soon after budding. However, the high anatomical variability of symptoms among unions from a given graft combination and at different places within a single graft interface makes the interpretation of microscopic observations difficult, especially in the early stages of graft development (Herrero, 1951; Mosse, 1958).

Therefore, we investigated the content of assimilating pigments and soluble sugars from leaves and soluble sugars distribution around the union. The results were interpreted in accordance with the anatomic aspects of graft union of four pear cultivars grafted on two rootstocks (*C. oblonga* and *P. sativa*). Experiments were carried out taking into account the research work on cultivars different response to the main pear disease (*Erwinia amylovora*), the pest attack (*Psylla* sp.). Also, the influence of rootstock genotypes on pear cultivars under Romanian pedoclimatic and agro-technical conditions was also take into account (Sestras *et al.*, 2008; Sestras *et al.*, 2009).

Experimental results (Tab. 1) revealed differences in the total amount of assimilating pigments between the variants of each cultivar ('Comtesse de Paris'/*C. oblonga*-2.55 mg g^{-1}, 'Comtesse de Raris'/*P. sativa*-1.84 mg g^{-1}) and between average values of variant I (2.34 mg g^{-1}) and variant II (1.94 mg g^{-1}). The rootstocks themselves are also characterized by different values of the total assimilating pigments content-3.69 mg g^{-1} (*Cydonia oblonga*) and 2.32 mg g^{-1} (*Pyrus sativa*). It is known that the rootstock genotype influences the graft growth, development and physiology (Sestras *et al.*, 2009). From this point of view, one can highlight how partners interact in these two types of combinations by showing the ratio of assimilating pigments content between grafted cultivar and its ungrafted rootstock. Therefore, the ratio of the total amount of assimilating pigments obtained from the average value of variant I (cultivars grafted on *C. oblonga*) and the ungrafted rootstock of *C. oblonga* was 0.68 (Tab. 1). Instead, the cultivars grafted on *Pyrus sativa* (average value of variant II) showed a ratio equal to 0.84, significantly higher compared with the cultivars grafted on *C. oblonga*. Similar differences were found on each cultivar of both variants. Since combinations known as compatible in Variant II were used, one would say that a higher ratio, closer to 1.00, shows a greater similarity between grafts and stock photosynthetic apparatus. Instead, lower values indicate significant differences between partners. Therefore, one could find that this ratio reflects the degree of affinity between scion and rootstock. In other words, as the ratio decreases the affinity between scion and rootstock might go down as well, revealing an increasing degree of difference between partners on anatomical, physiological or genetic level. Under these conditions, the grafted tree system suf-

Tab. 1. Average values of assimilating pigments content into the leaves, over four generations, of first year grafted pear trees (years 2006-2009)

	Type of grafting	Chl. *a* mg g^{-1} (DW)	Chl. *b* mg g^{-1} (DW)	Car. mg g^{-1} (DW)	Σ	Chl./Car. ratio	Chl.*b*/car. ratio	Σ/ΣC ratio
Variant I	'Comtesse de Paris'/*C. oblonga*	1.48±0.11	0.42±0.03	0.65±0.05	2.55	2.9:1	0.6	0.69
	'Williams'/*C. oblonga*	1.38±0.11	0.43±0.04	0.42±0.04	2.23	4.3:1	1.0	0.60
	'Trivale'/*C. oblonga*	1.55±0.12	0.45±0.03	0.45±0.04	2.45	4.4:1	1.0	0.66
	'Triumf'/*C. oblonga*	1.35±0.11	0.39±0.03	0.40±0.03	2.13	4.4:1	1.0	0.58
	Average value variant I	1.44	0.42	0.48	2.34	3.9:1	0.9	0.63
C	*Cydonia oblonga* BN 70	2.11±0.12	0.82±0.04	0.76±0.03	3.69	3.9:1	1.1	---
	Type of grafting	Chl. *a* mg g^{-1} (DW)	Chl. *b* mg g^{-1} (DW)	Car. mg g^{-1} (DW)	Σ	Chl./Car. ratio	Chl.*b*/car. ratio	Σ/ΣP ratio
Variant II	'Comtesse de Paris'/*P. sativa*	1.14±0.10	0.36±0.03	0.33±0.02	1.84	4.5:1	1.1	0.79
	'Williams'/*P. sativa*	1.29±0.10	0.49±0.04	0.49±0.03	2.27	3.6:1	1.0	0.98
	'Trivale'/*P. sativa*	1.04±0.09	0.34±0.02	0.34±0.03	1.72	4.1:1	1.1	0.74
	'Triumf'/*P. sativa*	1.19±0.10	0.38±0.03	0.36±0.02	1.93	4.4:1	1.0	0.83
	Average value variant II	1.17	0.39	0.38	1.94	4.1:1	1.0	0.84
P	*Pyrus sativa*	0.96±0.11	0.71±0.03	0.65±0.03	2.32	2.6:1	1.1	---

Each value is shown as the mean ± S. D. of 24 samples; D W-dry weight C-*Cydonia oblonga*, P-*Pyrus sativa*

fers an increasingly biological stress, leading to the rejection between graft and stock.

Just as chlorophyll loss is associated to environmental stress, the variation of total chlorophyll/carotenoids ratio may be a good indicator of stress in plants (Hendry and Price, 1993). The biological stress due to anatomical, physiological or genetic differences between scion and rootstock of grafted trees can also cause the decrease of the assimilating pigment content. It is known that a quantosome contains 160 chlorophyll *a* molecules + 70 chlorophyll *b* molecules + 48 molecules of carotenoids etc. (Park and Biggins, 1964) in a theoretic chlorophylls/carotenoids ratio of 4.8:1 and chlorophyll *b*/carotenoids ratio of 1.5:1. Although chlorophyll *b* is the major accessory light-absorbing pigment in the majority of eukaryotic photosynthetic organisms, with the exception of the red and brown algae, we obtained some higher values of carotenoids. We know that carotenoids have several well-documented essential functions in photosynthetic systems. First, they are accessory pigments in the collection of light, absorbing light and transferring energy to a chlorophyll-type pigment. Most antenna complexes contain carotenoids (Blankenship, 2002). Second, carotenoids have a function in a process called photoprotection. Carotenoids rapidly quench triplet excited states of chlorophylls before they can react with oxygen to form the highly reactive and damaging excited singlet state of oxygen. They also quench the singlet oxygen if it is somehow formed. Finally, carotenoids have been shown to be involved in the regulation of energy transfer in antennas (Blankenship, 2002).

The analysis results show that in cultivars/*C. oblonga* combinations the ratio between chlorophylls (*a* + *b*) and carotenoids is reduced on average with 18.75% and in cultivars/*P. sativa* combinations the ratio decreased with 14.58% compared to theoretic data. In addition, the ratio of chlorophyll *b*/carotenoids in both combinations is 40% and 33.33% lower, respectively. According to the literature, the change in the chlorophylls (*a, b*)/carotenoids ratio in favor of carotenoids can be explained by the trees need for photoprotection. These assumptions are confirmed by pronounced continental climate in the North-Eastern region of Romania, where winters are cold and summers are warm, with a dry character. However, some growth in carotenoids content was found in both variants (I and II) of the experiment and also in *C. oblonga* and *P. sativa* rootstocks. This fact leads us to conclude that the carotenoids content of the studied cultivars is not strongly influenced by compatibility between scion and rootstock, but rather by environmental factors.

Within this experiment, we also determined the soluble carbohydrates in leaves to highlight a possible correlation between variations of assimilating pigments and soluble sugars contents (Tab. 2, Fig. 1) depending on the type

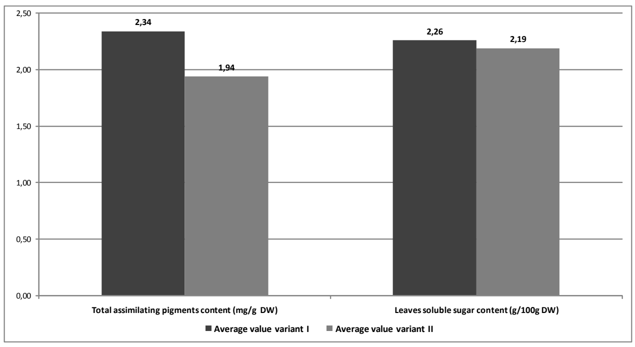

Fig. 1. Average values of assimilating pigments and soluble sugars from leaves, over four generations, of first year grafted pear trees (years 2006-2009)

Tab. 2. Average values of soluble sugars content into the leaves and the union, over four generations, of first year grafted pear trees (years 2006-2009)

	Type of grafting	Leaves (g 100 g^{-1} DW)	Above union (mg g^{-1} DW)	The union (mg g^{-1} DW)	Below union (mg g^{-1} DW)
Variant I	'Comtesse de Paris'/*C. oblonga*	3.19±0.23	3.51±0.26	5.30±0.37	5.02±0.31
	'Williams'/*C. oblonga*	2.16±0.20	3.53±0.21	7.14±0.42	5.97±0.34
	'Trivale'/*C. oblonga*	1.95±0.12	3.10±0.23	3.58±0.23	2.46±0.13
	'Triumf'/*C. oblonga*	1.74±0.13	2.78±0.18	4.96±0.32	3.03±0.17
Variant II	'Comtesse de Paris'/*P. sativa*	2.19±0.18	3.81±0.23	7.31±0.57	6.72±0.42
	'Williams'/*P. sativa*	1.97±0.14	3.34±0.17	3.92±0.25	3.08±0.16
	'Trivale'/*P. sativa*	2.21±0.17	4.14±0.31	4.88±0.33	5.64±0.23
	'Triumf'/*P. sativa*	2.41±0.19	2.06±0.15	7.56±0.49	5.56±0.35
	Average value variant I	2.26	3.23 (26%)	5.25 (42%)	4.12 (33%)
	Average value variant II	2.19	3.34 (23%)	5.92 (41%)	5.25 (36%)
	Cydonia oblonga BN 70	1.51±0.14	---	---	---
	Pyrus sativa	2.06±0.15	---	---	---

Each value is shown as the mean ± S. D. of 24 samples; D W – dry weight

of rootstock used. Analyzing the data presented in Tab. 2 one can see that, in both variants, there are no significant differences of soluble carbohydrates content. The average values between variants differ only with 3%.

Marini (1986) did not obtain a high correlation between chlorophyll content and photosynthesis rate. Accumulation of carbohydrates in leaves during photosynthesis is a common phenomenon which can be enhanced by several means including high photosynthesis rates, low translocation rates, or low sink demand (Neales and Incoll, 1968). In addition, it has been hypothesized that the accumulation of assimilates in an illuminated leaf may be responsible for a reduction in the net photosynthesis rate for that leaf. This 'end-product inhibition of photosynthesis' hypothesis was proposed over 100 years ago by Boussingault (1868) and was confirmed by many researchers over time (Neales and Incoll, 1968, Goldschmidt and Huber, 1992).

A conclusion drawn from the results showed that there is no need for a bigger content of assimilating pigments in order to have a higher photosynthesis rate and a high amount of carbohydrates in trees leaves. One may conclude that the concentration of soluble sugars in leaves depends on the cells osmotic pressure pattern and stress adaptation, rather than depending on the amount of assimilating pigments and the soluble carbohydrates distribution around the graft union highlights some differences in terms of carbohydrate content of the graft, the union and stock, though these differences are minor between variants. For pear cultivars engrafted on quince rootstock, the average values of soluble sugars are 26% above the union (in scion), 42% on the union and 33% below the union (in rootstock). The distribution of soluble sugars content in pear cultivars grafted on Pyrus sativa was as follows: 23% in scion, 41% in the union and 36% in rootstock. In both variants, the highest soluble sugars content was found at the union point. Thus, one cannot consider these results a marker for compatibility between scion and rootstock of first year grafted trees. As we mentioned above, the content of soluble sugars in leaves is rather a cultivars characteristic, with values close to the species featured values. Regarding the distribution of soluble carbohydrates around the union, we highlighted their retention on the union and in the superior part of the rootstock. The quantities of soluble carbohydrates are close in both compatible and incompatible combinations.

If we look carefully at some anatomical sections through the union of cultivar 'Comtesse de Paris' grafted on *C. oblonga* and *Pyrus sativa* seven weeks after grafting (Fig. 2 and Fig. 3), we can see that the scion and rootstock`s tissues were welded equally well on both rootstocks. In addition, we cannot observe any abnormality on the welding line: it is well defined, continuous, tissues are compact and colorful on both partners (scion and rootstock), without any necrosis area. Yet it's worth checking out the evolution of the grafted subject. For this purpose some additional observations were further made. It was found that the anatomical sections of the union after four years (Fig. 4 and Fig. 5) show a completely different picture. If the welding line between scion and rootstock for 'Comtesse de Paris'/*P. sativa* combination (Fig. 5) is almost undetectable, the one for 'Comtesse de Paris'/*C. oblonga* combination has become even more evident, with necrotic cells, clearly delimiting the scion and rootstock tissues. This leads to the weakening of the junction between grafts and rootstock, decreased resistance to mechanical force, and therefore the tree will break at this level. Moreover, the presence of this clear line of separation between scion and rootstock indicates that there is a disruption in continuity of vessels at this level. This disruption makes it difficult for the sap flow at this level, having negative consequences on tree growth and development.

Finally, as shown in Fig. 2 and Fig. 3, one can say that in the first months after grafting, callus formation and tis-

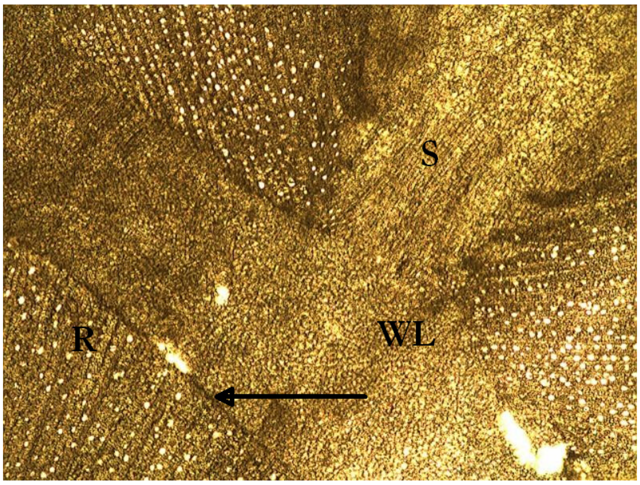

Fig. 2. Transversal section through union 7 weeks after grafting ('Comtesse de Paris'/*C. oblonga*); R-rootstock, S-scion, WL-welding line

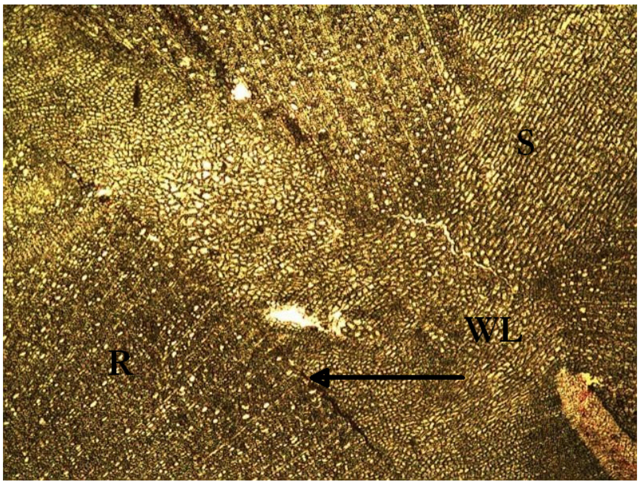

Fig. 3. Transversal section through union 7 weeks after grafting ('Comtesse de Paris'/*P. sativa*); R-rootstock, S-scion, WL-welding line

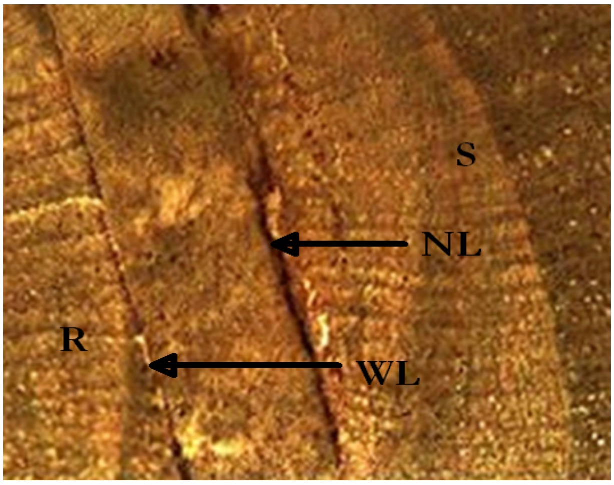

Fig. 4. Transversal section through union 4 years after grafting ('Comtesse de Paris'/*C. oblonga*); R-rootstock, S-scion, WL-welding line, NL-necrosis line between initial scion wood and scion wood differentiated after grafting

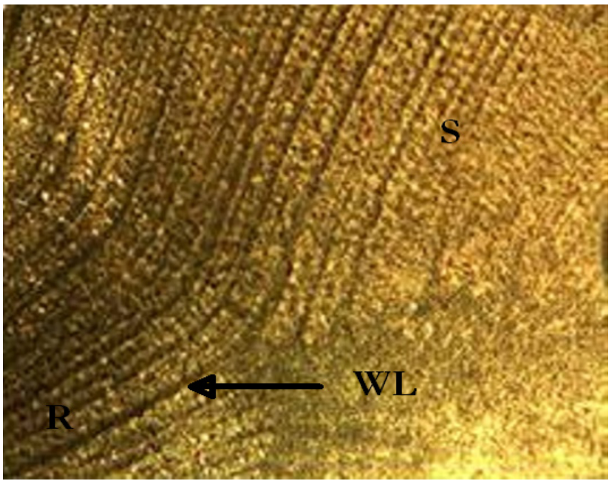

Fig. 5. Transversal section through union 4 years after grafting ('Comtesse de Paris'/*P. sativa*); R-rootstock, S-scion, WL-welding line

sue regeneration of the tested pear cultivars, grafted on *P. sativa* and *C. oblonga*, occurs normally. Also, the analysis results of soluble carbohydrate contents were similar in both variants in the first year of vegetation. This led us to conclude that the pear cultivars 'Comtesse de Paris', 'Williams', 'Trivale' and 'Triumph' show some grafting affinity for quince rootstock at the beginning of their common life. However, with time passing, a partial incompatibility phenomenon installs between these varieties of pear and quince rootstocks. The trees survive for several years, with severe repercussions on their growth, development and viability. Eventually these combinations fail because the trees themselves die.

Conclusions

The results obtained from this experiment highlighted the influence of grafting incompatibility on the total content of pigments in the following way: cultivars grafted onto a compatible rootstock had the content of the total assimilating pigments closer to the rootstock content, than those grafted onto an incompatible rootstock. Nevertheless, carotenoids amount is not dependent on the affinity between scion and rootstock but on environmental factors.

The amount of soluble sugars in leaves varies with pear cultivars but the values are close to the value of *P. sativa*. This fact leads one to believe that the content of soluble carbohydrates in leaves is a genetically determined characteristic of the species and cultivars. Regarding the distribution of soluble carbohydrates around the union, their retention on the union and in superior part of rootstock was highlighted. The same situation occurs in a similar manner in both variants, concluding that grafting itself could be an impediment of photoassimilates movement in grafted trees.

Considering the results of the analysis and anatomical sections through the union it can be seen that the pear cultivars 'Comtesse de Paris', 'Williams', 'Trivale' and 'Triumph' show some affinity for the quince rootstock at the beginning of their common life. However, with time passing, a partial incompatibility phenomenon installs, allowing trees to survive for several years, but with severe repercussions on their growth, development and life longevity.

Acknowledgments

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