

SUMMARY

of doctoral thesis entitled:

***„Influence of some physiological and biochemical processes upon
scion-rootstock association at fruit tree species,
that will help to increase agro productivity”***

realised by eng. Cristina ZLATI

Fruit growing is generally one of the main sectors of agriculture and especially of horticulture. And for this reason it represents one of the most intensified sectors of vegetal production, being on one of the top places as economic efficiency. One of the nursery production objectives is to obtain certified planting material concerning its authenticity, health and suitability for different culture systems. This material has to belong to recommended varieties and to be authorized for cultivation

Grafted trees represent a new organism, the two components - scion and rootstock being in a permanent and mutual interdependence. Researches in this field showed that rootstock has the most influence and this can be observed not only in scion growth and development dynamic but also on its morph-anatomical structure formation. The scion itself can be characterized by a big stability of its own metabolism and through this can influence the rootstock physiological and biochemical. Being a very complex phenomenon, the incompatibility is the result of numerous factors influence involved into tissue regeneration processes, so that a clear determination of both symptoms and causes that can be generated is quite difficult. Varieties genetic characteristics and even more, of the species, have a determinant role on incompatibility symptoms expression and sometimes can drove to the delay or the contrary, the early expression of these symptoms.

Even though, with all known methods, the early and accurate diagnosis of incompatibility causes is not totally possible. Knowing the compatibility degree between the two partners used to be determined and for the new varieties still is determined by ex situ experiences, in the nursery and later in the orchard, but some symptoms may occur even after long years after planting.

Compatibility problem is still insufficient known, being necessary to take into consideration this phenomenon at grafting and planting material producing. Because of the fact that till present are not known reliable methods of analysis, partners' compatibility will be evaluated only after grafting and long observations after planting.

Researches in the field shows that wide world, lately are used more and more, faster lab methods which allows aprioristic appreciation of compatibility degree.

Though, the main reason of the present research is the highlighting of the morph-anatomical aspects that appear at some fruit growing species with different degrees of compatibility with the chosen rootstocks and correlation with the changes that appear in grafted trees physiologic and biochemical metabolism in order to find out all the factors involved in incompatibility expression.

General objective of the PhD thesis is to undertake fundamental research, oriented to understanding the morph-physiologic, structural and biochemical changes and study of the possible mechanisms that appear at scion/rootstock association. The results would be useful in the better understanding of incompatibility phenomenon. Without a rigorous knowing of all these changes and without their correct understanding can't be elaborated *early detection methods* of the incompatibility between new grafting combination. Though, in order to find new methods and models for improving the results in early detection, there were necessary fundamental experiments, to study the processes that occur during bounding after grafting and plants monitoring years after planting. In this way, after consulting specific literature, first there were undertake own research on microscopy in order to determine the structural changes that appear in the grafting area. Another set of experiments were aimed on the determination of variation of some biochemical indicators as peroxides and catalase activity, chlorophyll pigments content, dry weight and carbohydrates content in the grafting area. There were made also investigations and observations of grafted plants growing and development processes.

For a better understanding of the influences upon grafting compatibility there will be taken into account some anatomic-morphological, physiologic and biochemical researches performed at young grafted trees from the following species: pear, plum and sweet cherry (at the scion, rootstock and in the grafted area) with the following specific objectives:

- observing the behaviour after grafting pear, plum and sweet cherry varieties on the two chosen rootstocks, in the ecopedoclimatic conditions of Eastern Moldavia;
- determination of the structural changes that appear at the grafting area;
- evaluation of scion-rootstock compatibility after callusing, in the correlation with anatomic-morphologic, physiologic and biochemical changes that appear;

- highlight the new aspects of compatibility and incompatibility at pear, plum and sweet cherry (forms, ways of expression), after callusing;
- determination of morph- anatomic- physiologic indicators for early detection, in the nursery, of incompatible combinations.

The thesis has 272 pages, where are included 86 tables, 122 figures and colour pictures (from which 50 are in original), conclusions and references that covers a number of 218 titles.

In chapter I there is made a presentation of fruit growing situation and development in the world and in Romania. Though, in 2007, in the world, fruit trees and bushes harvested area was of 47 143.638 thousand hectares, counting a total production of 499 711.349 thousand tones, with an average production of 10.6 tones per hectare. In Romania, harvested area was of 356.575 thousand hectare, with a production of 1 509.822 thousand tones, the average production being of 4.23 tones per hectare.

In chapter II are described the biological and technological particularities of each fruit growing specie taken into study, emphasising on the critical aspects.

Chapter III represents state of the art of the present subject, reviewing the incompatibility researches around the world. Thus, there are mentioned some citations since 1933 (Tukey H.B. and K.D. Brase) till present (Simkhada P.E., 2007; Dolgum O., 2008). There are highlighted form of manifestation of incompatibility phenomenon and the changes that appear.

In chapter IV are presented the ecological condition where the researches took place, reaching the conclusion that Iasi region, with an optimum thermic potential (multi annual average temperature of 9,6°C, multi annual average precipitations of 517.8 mm) is favourable for most of fruit growing species development.

In chapter V there is described the biological material used, research methods, chemical analysis, biometric measurements, determinations and observations there were made.

As biologic material was used four pear varieties: 'Curé', 'Euras', 'Countess of Paris' and 'Williams', grafted on quince BN 70 and pear seedling 'Harbuzești'; four plum varieties: 'Stanley', 'Centenar', 'Pescăruș' and 'Tuleu gras', grafted on wax cherry tree and Prunus seedling Renclod verde F; and four sweet cherry varieties: 'Germersdorf', 'Van', 'Stella' and 'Boambe de Cotnari', grafted on mahaleb and seedling cherry 'Bigarreau Jaune Dönissen'.

To achieve the objectives and to obtain fair scientific results concerning grafting compatibility of some pear, plum and sweet cherry varieties, there were used specific work methods, biometry and anatomic, physiologic and biochemical analysis. The observations and determination were made during 2005-2008.

In the nursery, scion-rootstock association behaviour was observed and registered with

biometry measurements, and in the laboratory there were made anatomic-morphologic observations and also physiologic and biochemical analysis.

Biometric measurements concerns: offshoots high, grafting area diameter, grafting success percentage determination etc. After these measurements were made there were set biometrical ratios between these elements as: there was determinate trunk transversal section surface and morphological index of thickening in the grafting area [Lucr. Șt. I.C.D.P. Pitești-Mărcăineni, 2006].

Anatomic-morphologic observations were made using fresh material, taken during vegetation period (July-August 2007). The probes consisted of fragments of 3-4 cm length, taken from 5 cm above and under grafting area and from the joining area. The probes were fixed ethylic alcohol 70°. Then, there were made transversal and longitudinal sections through the grafting area using CUT 6062 Slee Mainz microtome. On these micro sections there were made observations concerning: vessels orientation; the way of orientation, if they are straight, or presents involutions, if xylem fascicule is continuous or interrupted in the joining area; vessels frequency determination in transversal plan, comparing with the other anatomical elements (there was determined the number of vessels in the grafting area); the presence or the absence of lacunars area; parenchyma cells, if are differentiated or not (asymmetric cells or total loss); medullar rays width; distance between two rays; rays continuity or discontinuity; histological elements size determination (average diameter of xylem vessels); determination of grafting partners anatomic resemblance index [Lucr. Șt. I.C.D.P. Pitești-Mărcăineni, 2006].

Physiological analyses were used as indicator for synthesis potential and followed: chlorophyllian and carotenes pigments determination, dry weight.

Biochemical analyzes followed: carbohydrates and proteins content, the intensity of peroxidase and catalase activity. For biochemical determinations there were used stem fragments that were taken 5 cm above and under grafting area and also from the joining area, than there were fine grinded and extracted by homogenization during one hour in 1/10 ratio.

In chapter VI there are presented the own research to achieve the objectives and the results there were obtained.

In the frame of the objective concerning the determination the changes that appear at the grafted variants in the grafting area, there were some situations when there were observed growing and callusing defects but even though, trees seems to have a proper development. The explanation of this phenomenon could be the fact the medullar rays passes from rootstock to scion through areas with undifferentiated parenchyma or even lacunar areas when there is registered an improper joining.

Macroscopic observations emphasized the presence of some symptoms of anatomic-morphologic incompatibility at all three studied species, symptoms that were correlated with some disturbances in trees growing and development after grafting.

These symptoms refers to vegetative growth slowing down; low percentage of grafting success at incompatible combinations, differences in ale scion/rootstock diameter and tissues hypertrophies in the joining area; weak resistance in the joining area. Also, there could be observed xylem vessels discontinuity, at some combinations there could be observed the sinuous aspect of the vessels. Generally, at grafted plants vessels continuity is interrupted during grafting and hydro conductivity restoration is one of the most important factors in the proper functioning of the new plant. In case of the varieties with good compatibility with the rootstock vessels continuity restoration is almost complete, nutrient transport being practical the same with the one of the not grafted plants.

Concerning vessels diameter, these presented higher values at the probes taken from the scion than the ones from the rootstock (under the grafting area). These explain the fact that at incompatible combinations assimilates transport is much slowed down through the joining area and part of synthesised substances in leaves are accumulated in the scion, above the grafting area, and not stored in the roots. In this case, in the grafting area and above can be detected higher concentrations of carbohydrates, which is an indicator for determining scion-rootstock compatibility (especially starch), cited of many authors.

The presence of necrosis tissues was associated with starch accumulation in the tissues above the grafting area and its absence underneath provokes a deficit in the root system because of phloem degeneration. At some scion-rootstock combination there was observed more than in the phloem vessels and the unbalanced accumulation of this element was associated with incompatibility phenomenon.

At incompatible plum/wax cherry tree combinations, even though in most of the cases there were registered deviations from longitudinal vessels targeting, there was observed more at pear, the presence of necrotic spots and lacunar areas, vessels different diameter measured above and under grafting area, the presence of undifferentiated parenchyma and joining areas were separated of dark brown suberificated tissues.

Concerning the dry weight, we reached the conclusion that at the compatible combinations assimilated substances have a more uniform partition in the grafting area, with small differences above and under joining area. At the combinations with lower compatibility, higher quantities of carbohydrates, starch and mineral substances were accumulated in the scion while the root system and the stock stem have lower reserves. Due to a weak accumulation of reserve substances in rootstocks of incompatible combinations was put on the brake of translocation of these substances through the joining area. Thus, for roots growth of these combinations remained lower reserve substances and therefore can be assumed the hampering of trees activity.

Examining data on the concentration of soluble carbohydrates in the grafting area could be found a general trend of increased carbohydrate content in the grafted area and the scion at the point of grafting. At plum varieties, medium carbohydrate content under the grafted area was 1.24 times lower than in the grafted area and 1.40 times lower than reported above the grafting point. Large quantity of carbohydrates above the joining line shows that the migration into their stock is made more difficult.

Carbohydrates accumulation in the lower part of the trunk explains an increase of scion growth which, above the joining line has an obviously bigger size diameter.

Determination of peroxidase and catalase enzymatic activity was used as a measure for assessing the existing level of stress-induced grafting, grafted plant capacity to respond adequately to this biochemical factor which reflect physiological state of the plant.

Measurements and determinations of the pear varieties, but also those made on plum and sweet cherry have shown an increased enzymatic activity in the grafted area, and above, highlighting the close correlation with the rootstocks were grafted. Being studied in terms of spatial distribution of enzymes, in most cases, peroxidase activity has been the greatest intensity at the point of grafting and above the grafting point. This is in direct correlation with the results obtained in case of carbohydrate content. Because carbohydrates are the respiratory substrate is explained the higher enzymatic activity at the grafting line. This may be one of the reasons justifying the higher activity of peroxidase in the scion compared with the area under the grafting area.

If we analyze the data on determining catalase activity at pear varieties grafted on quince, in the grafting area and compare results obtained from samples above the grafting point, we can see that enzyme activity was more intense at the grafted line level, being de 1.75 times higher than above the grafting zone.

In plum, the presence of a more intense catalase activity in varieties with good compatibility with wax cherry tree, as Stanley, reveals the presence of lignifications processes in this area, which, also correlates with anatomic-morphological observations, when was observed the restoration of vessels continuity throughout all the grafting area.

In the last part of the thesis there are presented general conclusions and recommendations that suggest the development of a program to assess the degree of compatibility between scion and rootstocks for new obtained varieties, using biochemical and checking the results using grafting partner's anatomical resemblance index and morphological thickening index.