SUMMARY OF WORKS
2009

Development of biological models consisting of graft -rootstock combinations produced in vitro and used to study the phenomenon of incompatibility not limited to the establishment of efficient methodologies for the production of grafted trees in vitro and optimization of micrografted plant cultivation conditions. Besides it is also necessary to establish similarity of anatomical morphological, physiological and biochemical processes taking place at the grafting point of both biological models.

In this respect, over the current project phase we plan to characterized graft clamping process and the following growth and development process of in vivo and in vitro grafted plants from physiological and biochemical viewpoint and determining similarity shells at two models of biological processes.

The used biological material was represented by varieties of the sort Pyrus (Triumph, Trivale, Williams and Paris Countess) incompatible with Cydonia oblonga. Of these varieties were used to determine morpho -anatomical, physiological and biochemical

In spring (April) of 2009, at 7 months after grafting was found a catch rate which ranged between 93.2 and 97.6% in the pear varieties grafted on quince and at the same cultvar grafted on Pyrus sativa

1. Morphometric aspects of growth processes of pear varieties grafted in vivo

Morphological sections made it in three different stem zones was relived sundries grafting zone structural abnormalities for varieties with a low affinity capability. We can observe wood desultory, undifferentiated parenchyma layers between scion and rootstock and in some cases parenchyma with suber and necrosis. At compatible combinations Cure/ Cydonia oblonga the grafting zone has a satisfactory development, with wood tissue completely developed, but with deviations from radial structure of xylem vessel due to the engrafting process.

The highest diameter between pear varieties was recorded at Comtese de Paris 8.7 µm while. The rootstock vessel diameter was smaller then scion, (6.05 – 7.43 µm at pear). The only exception is represented by the Triumf variety which have the xylem vessel smaller in scion then engrafting point and rootstock.

Making a comparison between xylem vessel from rootstocks and scions at pears with different compatibility grades we can see at Cure variety, compatible with quince, the difference between vessel diameter in all three stem parts are very small (0.39). The other varieties wich present engrafting incompatibility recorded differences are greater (1.1 – 2.37).

For understanding differences between xylem vessel at scions, rootstock level and their role in incompatibility phenomenon it was made correlation coefficient between partners vessel dimensions and a high correlation between point -graft diameter and graft and rootstock diameters, r = 0.8811 and r= 0.9252., you can see that graft point diameter is most depending to rootstock diameter than to scion's.

Noteworthy is that although there are clear correlations between graft diameter and diameter section of scion and rootstock, but value of ratio between diameter of scion / rootstock does not correlate with graft diameter point (r= 0.1546). Furthermore this report can be correlated with percentage graft succes in this regard is found to inverse correlation with r = -0.7084.

Table 1.

Parametrii morfometrici ai soiurilor de păr altoiţi pe diferiţi portaltoi

Soiul	Portaltoi	prindere (%)	L altoi (cm)	D portaltoi (mm)	D. altoi (mm)	D punct altoire (mm)	D. altoi/portaltoi (mm)
mai							
Cure	*C.oblonga*	85.32	45.1	14.6	7.1	19.5	0.48
*C.*de Paris	*C.oblonga*	67.3	42.3	14.2	6.1	20.4	0.42
U. bosc	*C.oblonga*	71.21	53.8	14.4	8.2	20.35	0.56
Williams	*C.oblonga*	74.19	15.8	15.8	8.55	23.95	0.54
Cure	*Par franc*	93.2	41.4	10.4	5.7	15.4	0.54
*C.*de Paris	*Par franc*	94.4	27.5	11.5	6.3	16.8	0.54
U. bosc	*Par franc*	97.6	46.8	15.8	7.5	20.6	0.47
Williams	*Par franc*	95.8	31.3	10.7	6.1	17.8	0.57
iunie							
Cure	*C.oblonga*	85.32	66.6	13.85	7.95	19.5	0.57
*C.*de Paris	*C.oblonga*	67.3	61.8	13.9	7.1	20.4	0.51
U. bosc	*C.oblonga*	74.19	15.8	15.4	9.55	23.95	0.62
Williams	*C.oblonga*	77.61	54.1	17	11.8	24.8	0.69
Cure	*Par franc*	92.2	52.4	11.4	6.7	15.4	0.58
*C.*de Paris	*Par franc*	91.4	48.5	12.5	7.3	16.8	0.59
U. bosc	*Par franc*	97.6	67.8	16.8	8.5	20.6	0.50
Williams	*Par franc*	95.8	44.3	11.7	7.1	17.8	0.61
iulie							
Cure	*C.oblonga*	85.32	107.4	19.34	18.57	30.76	0.96
*C.*de Paris	*C.oblonga*	67.3	103.6	16.02	13.47	26.61	0.84
U. bosc	*C.oblonga*	74.19	104.7	16.45	13.76	26.66	0.83
Williams	*C.oblonga*	77.61	112.5	18.91	13.84	25.31	0.73
Cure	*PF*	92.2	101.5	18.11	17.65	24.71	0.97
*C.*de Paris	*PF*	91.4	91.3	14.89	12.78	19.69	0.85
U. bosc	*PF*	97.6	92.4	17.21	16.22	23.47	0.94
Williams	*PF*	95.8	98.8	14.78	12.71	17.62	0.85
august							
Cure	*C.oblonga*	85.32	110.4	21.34	20.57	32.76	0.96
*C.*de Paris	*C.oblonga*	67.3	109.6	18.02	15,47	28.61	0.85
U. bosc	*C.oblonga*	74.19	112.8	18.45	15.76	28.66	0.85
Williams	*C.oblonga*	77.61	122.5	21.91	15.84	27.31	0.72
Cure	*PF*	92.2	104.5	20.11	19.65	26.71	0.97
*C.*de Paris	*PF*	91.4	101.6	16.89	13.78	21.69	0.81
U. bosc	*PF*	97.6	98.4	19.21	17.22	24.47	0.89
Williams	*PF*	95.8	108.5	16.78	14.71	19.62	0.87

In conclusion, we can notice that the evolution of grafted trees stem diameter reflects a direct influence of rootstock growth process on scion growth and development and to some extent and the diameter of the graft point, but differences of vigor of scion and rootstock diameter can be correlated with the grafting point. However as the existing differences in graft and rootstock are both higher catch rate is lower grafted trees.

2. Regular morpho-anatomical sections through the point of grafting trees with different degree of affinity

One of the theories on the causes of the phenomenon of incompatibility suggests that these differences in the size of the graft and rootstock conductive vessels. To this end we proposed anatomic-morphological analysis of the grafting area linking xylem size vessels with some abnormalities occurring in combination with low-affinity. In this anatomical sections were made 5 cm below the grafting point, in the grafting point and 5 cm above the grafting point.

Microscopic observations were performed on sections made from the point of hair grafting varieties with different degrees of compatibility with rootstock. Microscopic preparations to carry out the grafting point was fixed in alcohol, sectioned with microtome, and double stained with ruthenium red and methylene blue (Fig. 1).

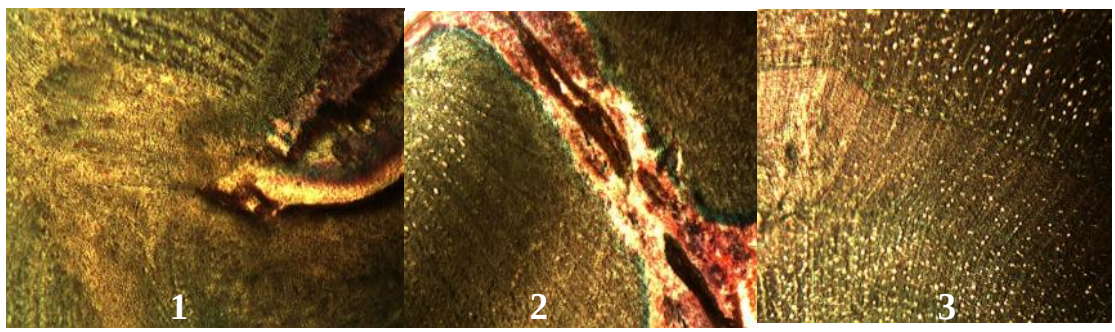

Fig. 1. Anatomically aspects of grafting incompatibility symptoms .
(1 -Suberification and tissue necrosis in combination bills Willams / C. oblonga;
2 – Invagination bark tissue between the scion and rootstock at Comptesse de Paris/ C . oblonga combination
3 – Success of grqfting in combination welding Curé / C. oblonga in combination)

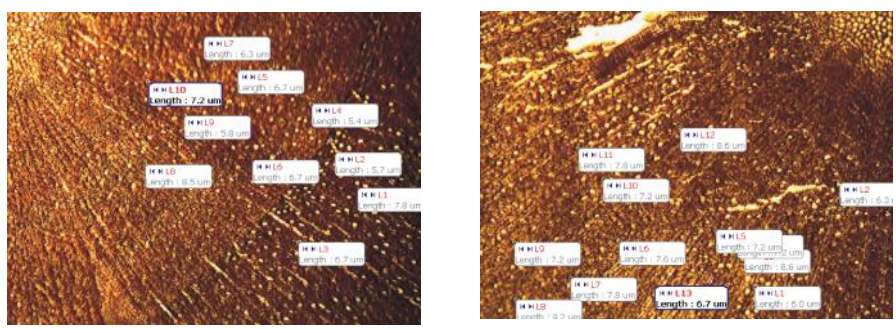

Fig.2. Xylemic vessel diameter in the scion and rootstock at scion Comptesse de Paris grafted on quince

Incompatible combinations were found discontinuities of wood, sized undifferentiated parenchymatous tissue between scion and rootstock and in some cases layers of parenchymatous necrotic tissue.

Alt the compatible associations Curé / *C. oblonga* accretion zone between scion and rootstock had a satisfactory development, with fully differentiated tissue with lemons, but with deviations from the radial stru cture of the location of xylemic vessels due to the grafting process (Fig. 2)

It can be observed that in all combinations studied, rootstock diameter vessels were significantly lower than scion's. The only exception is the Triumph variety which vessels were smaller in diameter than the rootstock and scion in the grafting point.

At incompatible varieties of pear was found a big difference between scion and rootstock vessel sizes comparing with differences found at compatible combinations.

It can be concluded that on the pear species, difference between vessels diameter can be considered an anatomical marker for incompatibility phenomenon (Tab.2).

Table.2.

Leading vessel sizes at the scion, rootstock and grafting point at some pear varieties grafted on quince

Scion's name	Diameter			Area		
	Scion	Rootstock	Grafted point	Scion	Rootstock	Grafted point
Curé	7,04	7,43	7,99	38,95	43,34	50,05
Comptesse de Paris	8,70	6,33	6,89	59,35	31,45	37,21
Untoasa Bosc	5,38	7,41	6,61	22,72	43,1	34,3
Willams	8,21	7,11	7,68	52,85	39,67	46,30

3. Comparative studies on physiological and biochemical processes taking place at the grafting point of pear /quince combinations produced in vivo and in vitro

This objective was aimed at determining the physiological indices (assimilatory pigment content and stomatal density) and biochemical (enzymatic activity, protein content, phenols, carbohydrates) at the grafted trees in the field and produced by in vitro micrografting.

Analyzing the results obtained and presented in the table 3 is apparent that the micrografted trees the leaf structure is less completed than at the trees grafted in the field, so in vitro produced plants has lower total pigment content but with a higher stomatal density than those produced in vivo.

These data show a more juvenil leaf of in vitro grafted trees than those produced in vivo.. Also it can see a higher enzymatic activity and increased total protein content in microgafted plants, but a lower content of carbohydrates and polyphenols. These data can ascribe a lower photosynthetic ratio and a lower intensity of secondary metabolism.

On the other hand in the variation of carbohydrates, proteins and polyphenols content in the scion, rootstock and grafting point it can observe the same trends that can fiind at the in vivo grafted trees, so the phenomena related to tissue regeneration at the graft are similar in both biological models. In conclusion, although the degree of maturatio n of

tissues and the growth and development of grafted plants in vivo and in vitro shows differensis , however in vitro grafted plants go through same stages of development and physiological and biochemical processes show similarity, entitle us to conside r this biological model usable to studing a graft incompatibility fenomenon.

Table 3

Variation of biochemical indicators in some varieties seem grafted on quince

	Total pigments	**Stomata's nr. /mm^2**	**Peroxides activity**	**Total protein**	**Sugars content**	**Total polyphenols**
In vivo						
Curé						
Scion	2,891	178.34	0.5029	78,1	0.4370	74.52
Rootstock			0.1081	62,5	0.7471	74,67
Grafted point			0.2351	87,5	0.8795	81,98
Comptesse de Paris						
Scion	2,818	184,12	0.1457	75,4	0.8067	102.22
Rootstock			0.5264	82,8	0.8413	65,27
Grafted point			0.2870	79,3	0.8136	176.86
Untoasa Bosc						
Scion	2,974	126,4	0.7895	73,4	0.5472	96,05
Rootstock			0.2961	65	0.8137	62,57
Grafted point			0.4559	88,4	1.2653	134,43
Willams						
Scion	2,740	189,37	1.1750	90,6	0.7236	100.44
Rootstock			0.8763	76,6	1.0314	52,87
Grafted point			0.8319	70,3	1.487	153,73
In vitro						
Curé						
Scion	1,983	237,14	0.7458	121.4	0.3125	74.11
Rootstock			0.3514	98.6	0.5421	81.24
Grafted point			0.4892	113.5	0.5935	88.16
Comptesse de Paris						
Scion	2,014	285,37	0.6524	124.18	0.2857	84.21
Rootstock			0.5685	78.48	0.5942	68.54
Grafted point			0.8725	135.89	0.5658	89.46
Untoasa bosc						
Scion	2,324	305,27	0.7958	111.2	0.6864	84.28
Rootstock			0.3533	65.6	0.5654	75.21
Grafted point			0.6562	125.6	0.8551	63.88
Willams						
Scion	2,236	288,91	0.9946	136.9	0.2653	99.51
Rootstock			0.6154	102.4	0.4244	89.12
Grafted point			0.8786	145.3	0.7414	104.31

The results from the *Pyrus* species entitled us to consider useful extension experiments next year on two other species of trees : plum and apricot, in the aim to verifying in other grafted combinations.

For accomplish of this objective 4 plum cultivars (Stenley, Tuleu timpuriu, Gras ameliorat si Centenar) and 4 apricot cultivars (Tudor, NJA 42, Umberto si Goldgich) has been grafted in August using the chip budding method, on *Prunus cerasifera* , P.F. Renclod verde si *Prunus armeniaca* rootstocks.

Technology and for standard maintenance in the first work field were applied, it was monitored healthy of the trees, grafting success percent and samples were harvested for morphological analysis anatomical point of grafting.

Simultaneously we tested a protocol for obtaining a graft technique of micropropagation for genotyping *in vitro* combinations. Axillary buds from the 8 varieties of plum and apricot were sterilized in 70% ethanol 2 min and 25 min in 1% sodium hypochlorite, then washed three times with sterile distilled water. Apical meristem were excised under a microscope and transferred to culture medium.

Were tested four culture media for apical meristems: MS, WP, ½ each B5 and MS medium was supplemented with 3% sucrose, 0.7% agar, and 1 mg / l BAP. Environment was divided into 10 ml tubes every 20 min and autoclaved at 121 °C. In each tube was placed one explant. After 30 days of culture was appreciated surviving rate of explants and shoot length.

MS medium was supplemented with various cytokinine: BAP, zeatina and kinetina a concentration of 0.5, 1, 1.5 and 2 mg / l. For each type of medium were used 15 explants. After 30 days of culture was estimated survival rate and length of the shoots.

Following presented data in the table below you can see that the WP culture medium of explants survival rate was 100%. It was found that optimal proliferation of shoots from *P. armeniaca* WP is medium, MS medium caused necrosis and vitrification of shoots after several subculturing. Snir (1982) show that the axillary buds of variety 'Caniro' were successfully grown *in vitro* on a medium consisting of Knop solution. (Sugiura et al.). The 1986 reported good results using culture medium MS1 / 2 and WP. However literature agrees on the fact that MS medium not conducive to developing axillary buds apricot due to high concentrations of nitrogen.

	Basal culture medium	Rate of surviving (%)	Soots dimension (mm)
1	WP +0,5 mg/l AIA+1 mg/l BAP	100	9,9
2	MS+0,5 mg/l AIA+1 mg/l BAP	66	4,7
3	1/2MS+0,5 mg/l AIA+1 mg/l BAP	86	4,4
4	B5+0,5 mg/l AIA+1 mg/l BAP	93	3,1

Cytokinins had different effect: 100% rate recorded a BAP and BA, 93.3% zeatina and K. In fact only 80% BA or BAP are most effective for induction and proliferation caulinare cultures.

	Basal culture medium	Rate of surviving (%)	Soots dimension (mm)
1	½ MS + 1mg/lBA	100	9,9
2	½ MS + 1mg/lBAP	100	6,6
3	½ MS + 1mg/lK	80	2,7
4	½ MS + 1mg/lZEA	93	4,6

Further subculturing the shoots on medium containing K leads to their vitrification. Zeatina and BAP had the most pronounced effect on proliferation of shoots

	Cytokinin	Conc.	Shoots number	Shoots dimension
1	BAP	0,5 1 2	3,1 6,3 4,5	7.7 6.7 5.9
2	K	0,5 1 2	1,3 1,4 1,1	11.3 13.8 26.3
3	ZEA	0,5 1 2	1,7 2,1 1,9	18.2 15.7 11.6

Experiments showed that there shoots during different stages of development require particular conditions of culture especially different balance of phytohormones. In the first stage, the apical meristem culture initiation of new bud formation is under the cytokinins influence. But very seldom endogenous cytokinins are sufficient for initiation of this process. In this case the environmental supplementation with relatively low concentrations cytokinins is mandatory. Addition of auxins, especially when is using large explants, it is not mandatory because the caulinar apexes are one of the most important offices of auxns biosynthesis and explants not lack this kind of phytohormons. If we add auxins it is recommended to use a weaker, like AIA or AIB, and not 2.4 D in low concentrations up to 1 mg / l. In the next stage of development is the proliferation of shoots for existing and generate a large number of adventitious buds. This goal is achieved through increased contribution of cytokinins in culture medium, which reduces apical dominance and stimulate adventitious bud emergence. However as can be seen from tab. 2 cytokinins increasing concentration over a certain threshold, usually above the limit of 1 mg / l in the studied species leads to decrease the number of generated shoots.